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# Journal of DENTAL RESEARCH

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## THE EDITOR'S VIEWPOINT

The very useful study of Prombo and Tilden (p. 108) on the efficacy of different disinfectants against streptococci, pneumococci, and tubercle bacilli should offer a guide to selection of the misnamed "sterilizing agents" for instruments. While iodine and saponated cresol were effective against all three organisms and a special 3 per cent formaldehyde solution and quaternary ammonium compounds were fairly effective, some favorites, such as phenols, mercurials, and alcohols, were not as useful as is generally assumed. The saponated cresol and 3 per cent formaldehyde is given first place, with "DC 12" (one per cent) following. Metaphen Disinfecting Solution is effective against streptococci.

Baume (p. 123) has studied the deciduous dental arches of 30 children during various stages of development, and concludes that there are two consistent morphological arch forms of deciduous dentures, those which were congenitally spaced and those which were congenitally closed. Baume does not believe the present concept of physiological changes in the deciduous dental arches through spacing and mesial shifting of the mandibular teeth is an actual event in the development of the child. It would be well for this work to be repeated in a large number of children and the validity of the old concept or new concept definitely established. The presentation of Baume appears to be well substantiated by his data and illustrations. The findings should be of great importance to all interested in the growth of jaws, especially orthodontists.

Howell and Brudevold (p. 133), using an apparatus similar to that described by Howell and Manly, measured vertical forces used in chewing food with an artificial denture. They found the forces smaller than anticipated, the mean force on the first premolar being 0.4 kg., on the second premolar 1.0 kg., and on the first molar 1.9 to 2.6 kg., with a maximum of 7.2 kg. on the first molar when chewing raisins. The second premolar was used consistently at full capacity but the first premolar was used more with tougher than with softer foods. These studies should be considered by prosthetic students and their extension may supply important information for development of complete and partial denture technics. Periodontists and orthodontists should utilize this type of information, too.

Gruber (p. 137) presents the result of clinical trial of a new local anesthetic agent. It is claimed to be superior to procaine in its rapidity of action, profundity, selectivity, duration, and lack of side reactions. This may offer a great relief to the small group of dentists who are sensitive to procaine, as well as its promise as a superior drug.

Angell (p. 143) compared physical properties of gold alloy wire and stainless steel wire for orthodontic arches and springs. On account of the greater modulus of resilience of gold alloy wire, he considered it superior to stainles steel wire for arches and springs. Laboratory tests, in instances such as this, may be substituted for years of uncontrolled clinical observation.

Brucker (p. 148) found more decay in the second than in the first deciduous molars and more interproximal than occlusal caries in the second deciduous molars. Food accumulation and difficulty in cleaning appeared to be factors. Negro children appeared to be more resistant to caries in the deciduous molars than white children. More caries was found in those teeth among the children of better economic classes.

Hunt and Hoppert (p. 157) found the distribution of carious lesions in the lower molars of caries-susceptible and caries-resistant albino rats. As would be expected, carious lesions show a predilection for certain sites but an unexplained finding is a significant tendency for more decay in the lower right than in the lower left molars. There is more evidence of this in the susceptible than in the resistant strain. Are the animals predominantly right-hand chewers or is this the long shot where the one-in-a-million probability paid off? The data should be of value to other laboratories and it would be of interest to learn if their rats also show the dextral distribution of caries.

Sullivan and Storvick (p. 165) analyzed the salivas of 574 college freshmen on whom clinical caries studies had been made. They found significant correlation between DMF and starch hydrolyzing time, lactobacillus counts, pH and buffer capacity but not between DMF and ammonia nitrogen. It is interesting that they found a highly significant relationship between DMF and salivary lactobacilli, as DMF is a record of caries history and the lactobacilli indicate present caries activity. It may have been fortuitous that in this group those with many cavities still had active carious processes. The age of the group may have been a factor here and in older individuals the activity may be reduced but of course the lesions will remain. Correlated studies such as this may afford useful basic data.

Sullivan and Storvick (p. 173) found positive correlation between Snyder tests and salivary lactobacillus counts and negative correlation between ammonia nitrogen and both of these tests. Positive correlation between salivary pH and buffering capacity was also noted. Starch hydrolyzing time did not show significant correlation with the other tests. It is unfortunate the clinical caries activity is not reported with these other data. Further evaluation of the various laboratory tests for caries activity are needed but they should be compared with the clinical picture.

Dannenberg and Bibby (p. 177) report a fundamental study of the effect of zinc chloride and potassium ferrocyanide on proteolysis. This work is long overdue on the basis of the recommendation of these agents for dental caries control. Although inhibition of proteolysis is the basis of the recommendation of these agents for caries prophylaxis, the chemicals actually increased proteolysis. Further, this work indicates that calcium salts must be removed before proteolysis can occur. If zinc chloride and potassium ferrocyanide applied topically do reduce caries activity, it is apparently not due to their interferences with protein breakdown.

Muhler, Boyd, and Van Huysen (p. 182) examined the ability of certain compounds to inhibit acid solubility of enamel, dentin, and tricalcium phosphate, in vitro. They believe the method superior to that which indicated that lead fluoride was superior to sodium fluoride for topical application, a suggestion from in vitro study that was not confirmed by in vivo suggestions. The present method places stannous fluoride first in the group of compounds tested for reduction of acid solubility of tooth tissues, in vitro. Clinical trial of this must be conducted before any claims for practical superiority can be supported. Because of the commercialization of acidulated sodium fluoride solutions on the basis of in vitro evidence, later shown to be contrary to clinical results, we are inclined to limit publication of such studies in the future until clinical tests are presented.

Granados, Glavind, and Dam (p. 194), who have used the hamster as an animal for caries studies, had not observed the higher susceptibility of males claimed by several authors. They examined 64 newly weaned hamsters, most of them males, after 100 days on an experimental diet. No significant sex differences were observed. Are there genetic differences in the hamsters used at Rochester and Zoller Clinic that are the causes of this difference? The slight femaleness, as well as right-sided distribution of caries, in Hunt and Hoppert's albino rats suggest such a strain difference. All of these experimental studies on laboratory animals genetic factors may be highly important. For example, what role did they play in the one sex protection against caries reported by Hein and Schafer using water-soluble chlorophyll compounds?

Shaw, Shaffer, and Soldan (p. 197) document the postnatal development of the cotton rat's molar. This material is of value to those who intend using the cotton rat as an experimental animal. The authors also note that animals fed purified rations had later development of the molars than those fed natural foodstuffs, and discuss this point in relationship to caries. However, caution should be used in attributing caries to interference with growth processes.

Gorlin (p. 208) was unable to find pathologic signs of scurvy in the teeth and bones of mice fed glucoascorbic acid. Since glucoascorbic acid is one of the antimetabolites recently investigated and had been specifically described as producing a scurvylike condition in species which normally produce their own ascorbic acid, these findings are of some interest. We cannot afford to assume that the presence of a nutritionally required

substance assures that it will perform its function. The whole problem of antimetabolites should be borne in mind by all investigators of deficiency states, and, when necessary, studies, such as that of Gorlin, conducted to evaluate the possible role of the antimetabolites.

Pindborg (p. 212) fed rats on a diet deficient in vitamin E and containing succinyl-sulfathiazole, which presumably prevented synthesis in the intestines. Depigmentation of the incisors and the formation of "cysts" in the enamel organ are reported. If tocopherol (or feces from normal rats) was added to the diet, or if succinyl-sulfathiazole was omitted, the changes did not occur, indicating that vitamin E deficiency, in the white rat, may produce changes in the amelogenic apparatus and that vitamin E may be synthesized in the rat's intestine. The "cysts" observed are interesting. If they can be produced early in teeth that do not erupt continuously, would dentigerous cysts result, or are they self-limiting?

Burstone (p. 220) investigated the effect of irradiation by x-ray on the jaws of young developing mice. The odontoblasts were more sensitive than the ameloblasts. As might be expected, the state of histogenesis of the tooth largely determined the effect under equal amounts of irradiation. The earlier the irradiation, the more the resultant hard tissue formation resembled primitive odontomes. Irradiation of embryos has shown to produce gross anomalies; irradiation of the developing teeth produced anomalies of the teeth which we would interpret as odontomes. Retardation of basal bone development and acute inflammatory response in the enamel organ also were observed.

Sallay and Nador (p. 232) investigated the changes in the kallikrein content of the saliva. Kallikrein, a vasodilating hormone, is believed secreted by the pancreas and salivary glands. Using changes in blood pressure in dogs after injection of saliva and of commercially prepared kallikrein as criteria, the authors observed an increase in the kallikrein content of saliva with ageing of the individual and an increase in patients with periodontal disease provoked by constitutional factors (periodontosis). If one accepts the blood pressure changes as due to kallikrein and not to other toxic substances in the saliva of older individuals and those with periodontal disease, the findings must be considered quite significant in explaining the changes sometimes described as precocious senile atrophy.

Rosenthal and Winter (p. 237) tested the relative efficiency of penicillin and streptomycin solutions in controlling bacteria in the acute phases of Vincent's infection. These findings indicate that streptomycin is superior because it controls the bacteria more effectively and can be stored for longer periods of time. It must be recalled (1) that these antibiotics will act as antibacterial agents against only certain microorganisms and (2) that control of bacteria, alone, does not stop most gingival and periodontal diseases.

Dawson and Blagg (p. 240) to study further the antibacterial activity of saliva employed an aluminum oxide chromatographic column for separation. Using a spectrophotometric technic with plating, lysis and inhibition were differentiated. Two active principles, destroyed at 100° C. but able to tolerate wide changes in pH, were obtained. Preliminary studies indicate that phospholipids may be involved. Low titer of the antibacterial salivary principles found in malnourished and cholera-infected groups is suggested as a possible explanation of why epidemic cholera is limited to malnourished groups. Socioeconomic planners have long overlooked this factor in disease, believing that by purchase of medical care disease can be eliminated. Rather, they might look closer to their own field and realize that only by improving nutrition and living conditions can certain diseases be controlled.

H. B. G. R.

# EVALUATION OF DISINFECTANTS BY TESTS IN VIVO

MARJORIE PIERCE PROMBO, M.S., AND EVELYN B. TILDEN, PH.D.

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THE desirable properties of a disinfectant* have been discussed by McCulloch.[1] The properties which can be expected of a chemical solution may be summarized as follows: It should be soluble in water or treated in such a way as to render it soluble without affecting its germicidal properties. It should be relatively stable in solution. It should have a low surface tension, so that it will spread evenly over surfaces to be disinfected; it should not have too great an avidity for combination with organic matter, so that its action on bacteria is lost in this way. It should not corrode metals nor injure fabrics. Finally, and most important, it should be able actually to kill microorganisms, not merely to inhibit their growth.

Toxicity to bacterial protoplasm without toxic action on other cells is not possible in the case of disinfectants, but one can certainly avoid agents like mercuric chloride, which is extremely poisonous to higher cells without having any advantage over less toxic substances in germicidal activity. Cost must be considered, but the less expensive chemical may be found to have more germicidal action than the costly ones. Action on spores is hardly to be expected of chemical solutions which satisfy the desirable properties listed, for example, that of freedom from injury to metals and fabrics. Fortunately, however, few spore-forming bacteria are pathogenic; that some are is the chief reason why sterilization by steam or hot air is to be recommended rather than disinfection whenever feasible.

The evaluation of disinfectants has always been a difficult problem. The determination of the "phenol coefficient" is an artificial test, and its limitations have long been recognized. It is still the official test,[2] but the tendency among research workers at present is toward a procedure which simulates the conditions under which a disinfectant is to be used,[3-11] and which takes into account as many as possible of the factors favorable and unfavorable to its action. It was the need for this sort of procedure which prompted Nungester and Kempf[12] to devise the technic which they called the "infection-prevention test."

In the infection-prevention test, a highly virulent culture of either the pneumococcus or the hemolytic streptococcus is swabbed on the tail of an anesthetized mouse. The tail is exposed to the disinfectant under test, and a portion

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The data reported are taken from the thesis presented by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Bacteriology in Northwestern University.

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**Accepted Dental Remedies* (ed. 4, 1948) defined disinfectants as "agents which are useful in decreasing the bacterial or mold population on instruments and equipment." The unfortunate term "cold sterilization" is obviously not applicable to the treatment of instruments with chemical solutions.

of it, about a centimeter, is snipped off and implanted in the peritoneal cavity of the mouse. Death of a large percentage of the mice treated in this way, with recovery of the infecting microorganism from the heart's blood, indicates failure of the disinfectant. Nungester and Kempf showed that this method was two or three times as sensitive as a cultural one for detecting a few bacteria which escaped the action of the disinfectant.* Bacteriostasis, which is responsible for undeserved high ratings in the determination of the phenol coefficient, is reduced or eliminated when a test is made in vivo, since the disinfectant remaining on the bit of tail is diluted by the body fluids and removed from the site of introduction, while the number of bacteria present in the body remains the same. The hemolytic streptococcus and the pneumococcus were chosen as test organisms because they can be made highly virulent for mice.

Although our interest was in the disinfection of dental instruments rather than of the skin, the technic of Nungester and Kempf seemed well suited to our purpose, particularly if carried out with the hemolytic streptococcus, which is spread from person to person very commonly by healthy carriers[14] and therefore might be present on imperfectly disinfected dental instruments.

*Mycobacterium tuberculosis* is known to be resistant to certain chemicals which destroy other bacteria, for example, chlorine, acids and alkalies, and the mercurials.[15] It was not feasible to use the mouse tail test against *Myco. tuberculosis*, since the mouse is not very susceptible to this organism. We accordingly set up a series of tubes as in determination of the phenol coefficient, using a homogenized suspension of the tubercle bacillus, and after periods of exposure of 10, 20, and 30 minutes injected a suitable amount of the mixture intraperitoneally into the guinea pig.

## EXPERIMENTAL TECHNICS

*Mouse Tests*:

*Procedure.*—A mouse of approximately 30 grams body weight was anesthetized by the intraperitoneal injection of 0.1 ml. of 1 per cent pentobarbital sodium, U.S.P. After 1 to 2 cm. of the end of the tail had been swabbed with the culture,† using five firm strokes, the tail was immersed in the disinfectant, contained in a sterile serological tube of approximately 2.5 ml. capacity, for exactly 2 minutes. A portion of about 1 cm. was then snipped from the end of the tail and laid in a small sterile Petri plate. The cut end was tied to prevent bleeding. The test is best performed by two persons: one anesthetizes the mouse and prepares the tail; the other secures the mouse to an operating board, places over the animal a sterile cloth with a suitable small opening, and makes a very small incision, first in the skin and then in the muscle layer of the abdominal wall. Since the abdominal wall has been shaved and the area disinfected with tincture of iodine by the assistant before the injection of the anesthetic, the operator needs only to make the abdominal incision and insert the piece of tail into the peritoneal cavity. One stitch in the muscle and two or three in the skin

*One bacterium may occasionally be sufficient to induce infection, as shown in the case of the anthrax bacillus by Barber.[13]

†Swabs such as are used for throat cultures were placed singly in test tubes plugged with cotton and sterilized in the autoclave at 121° C. for 20 minutes.

are sufficient to close the incision. The instruments needed for each mouse are one pair of plain and one of tissue forceps, two scissors (one for cutting off the tail), and a hemostat. The needle used is a small curved surgical needle 2 cm. in length. Cotton thread No. 50 was found suitable for suturing.* The test was usually done with a stock of instruments sterilized by dry heat, but boiling for 10 minutes was found entirely satisfactory. Once proficiency in the technic had been acquired, no trouble was experienced from secondary infections or from deaths due to the operation.

*Culture.*—A standard procedure for maintaining uniform virulence of the test culture is perhaps the most important factor in the use of the infection-prevention technic. In the case of the pneumococcus, cultures may be made exceedingly virulent, so that 0.1 ml. of a $10^{-8}$ dilution of an 18- to 24-hour culture will kill mice in 48 hours when injected intraperitoneally. We found a titer of $10^{-6}$ satisfactory, and this could be maintained by biweekly passage. Several blood agar slants were inoculated from a pure culture isolated from a mouse moribund from pneumococcus infection and were incubated until good growth was obtained, then stored in the refrigerator. These slants served for a period not exceeding 2 weeks as the source of inoculum for brain heart infusion broth (Difco) containing 0.2 per cent dextrose and adjusted to pH 8.0. A culture 16 hours old, one part of which was diluted with 4 parts of broth before use, was apparently as satisfactory as the 6-hour culture used by Nungester and Kempf. When such a culture was titrated, 0.1 ml. of a $10^{-6}$ dilution was always fatal to mice in 48 hours. Titrations of virulence are particularly important if the experiments have been interrupted for an interval. The culture used was a Type I pneumococcus.†

The hemolytic streptococcus was strain C 203.‡ Its virulence was maintained in much the same way as that of the pneumococcus, except that the pH of the broth was 7.6, and 5 per cent defibrinated rabbit blood was added.§ The supernatant fluid, free from blood cells, was used in the test. Thus maintained, the culture had a constant virulence, so that 0.1 ml. of a $10^{-4}$ dilution intraperitoneally was invariably fatal in 24 hours.

*Period of exposure.*—Although the procedure calls for an exposure to the disinfectant of exactly 2 minutes, we found by keeping careful records that by the time the piece of tail was cut off, the stump ligated to prevent bleeding, and the mouse prepared, the time elapsed was approximately 4 minutes.**

*Recording of results.*—The mice were observed for a period of 2 weeks, and the heart's blood of those which died was streaked on blood agar plates. It was

*The needles were threaded and placed singly in small Petri plates (2.5 inches in diameter), which were sterilized by dry heat (160° C. for 90 minutes) in small tin fruit cans.

†Obtained through the courtesy of Dr. O. H. Robertson, Department of Medicine, University of Chicago, Chicago, Ill.

‡We are indebted for this culture to Dr. Eleanor Bliss, Department of Preventive Medicine, Johns Hopkins University School of Medicine.

§Several months after these experiments were discontinued, it was desired to carry out a class experiment. The culture had been passed through animals at monthly intervals, but virulence was low. By rapid passage from mouse to mouse at intervals of 6 to 8 hours virulence was built up again, but it was found advantageous to use mouse rather than rabbit blood in the broth medium.

** The Council on Dental Therapeutics recommends a period of at least 10 minutes for disinfecting instruments, hence the time used in our experiments is well within that needed to provide a factor of safety.

found desirable to use sodium azide in the concentration of 1:5,000 in the blood agar plates, because it suppresses the growth of post-mortem secondary invaders while not interfering with growth of streptococci or pneumococci.

*Guinea Pig Tests*:

*Procedure.*—Four and one-half millimeters of the dilutions of disinfectant were distributed into tubes such as are used in the standard phenol coefficient test (1 × 5½ inches) and placed in a water bath at 37° Centigrade. The suspension of *Myco. tuberculosis*, prepared as described below, was added by pipette. After 10, 20, and 30 minutes,* 0.1 ml. of each mixture was injected intraperitoneally into guinea pigs of approximately 300 grams weight, duplicate animals being used for each dilution and for each time interval. It was necessary to prepare a time chart in advance which would allow time for injection of the animals. Three to five controls received a similar dose of the bacteria which had been exposed only to physiological salt solution. After a period of 3 months, the guinea pigs were sacrificed. Gross examination at autopsy was usually sufficient for diagnosis of tuberculous infection, but histological examination of tissue sections was made in doubtful instances. We were fortunate in having the cooperation of Mr. John Kloeck,† then of the Chicago Municipal Tuberculosis Sanitarium, in the preliminary experiments, and we are indebted to him for the method used in making the homogenous suspension of *Myco. tuberculosis* described below, and for help in the autopsies in the early experiments. Mr. Kloeck also furnished the culture (H 37 RV) used in the preliminary experiments.‡

*Culture.*—A culture, 10 to 14 days old, of *Myco. tuberculosis* on Herrold's egg yolk medium was used. Flakes of culture were weighed on a microbalance (2 to 3 mg.),§ and this was ground in a large thick-walled test tube by a glass pestle made to fit the tube. To this 5 ml. of distilled water was added, and when the suspension of the tubercle bacilli was homogeneous, it was further diluted so that 1 ml. contained 0.01 mg. of culture. A further tenfold dilution was made by adding 0.5 ml. of culture to 4.5 ml. of the disinfectant. Each guinea pig received 0.0001 mg. of culture (0.1 ml.), except in one experiment, in which the dose was 0.001 mg.

In the guinea pig tests we found it necessary to detoxify some of the disinfectants after the tubercle bacilli had been exposed to them for the required period, but before injection into the animals. Iodine was detoxified by drawing into the syringe enough sodium thiosulfate to decolorize the iodine and injecting the animal with a correspondingly larger amount of the mixture. Formaldehyde was rendered nontoxic by adding to the solution in the syringe an equal amount of a solution of glycine containing 10 grams in 30 ml. and diluting with saline

*Since the 20- and 30-minute intervals gave the same results as the 10-minute one, the results in the charts are based on the 10-minute interval.

†Mr. Kloeck left for the Medical Clinic, Seattle, Wash., shortly after the completion of the first series of experiments.

‡In the later work, a culture of H 37 RV, which is used extensively for experimental work, was obtained from Dr. Guy Youmans, of Northwestern University Medical School.

§Dr. Marie Berg, of the Chemistry Department of Northwestern University Dental School, was kind enough to make these weighings.

to 1 ml., all of which was injected into the guinea pig. "DC 12" was treated with an equal volume of soap solution (1 per cent Ivory soap flakes) before injection.

*Chemicals tested.*—The following chemicals were obtained on the open market. In most instances the information regarding chemical structure and composition is available in the U. S. Pharmacopoeia, the National Formulary, The U. S. Dispensatory, or Accepted Dental Remedies. In case the chemical was not described in any of these sources, the description is taken from the label on the container.

Orthophenyl phenol crystals. (Sargent)

Orthophenyl phenol 13 per cent (Phenolor, Squibb. Contains 13 per cent soap and 3.8 per cent isopropyl alcohol by weight)

Nitromersol, N. F. (Metaphen, Abbott). An anhydride of 4-nitro-3-hydroxy-mercuri-ortho-cresol.

1:1,000 solution contains 2 Gm. nitromersol, 0.4 Gm. sodium hydroxide, and 4.25 Gm. of sodium carbonate monohydrate in 1,000 c.c. of distilled water.

1:2,500, List No. 3503, 1 part dissolved in 2,500 parts of water containing 0.33 per cent each of sodium bicarbonate and sodium carbonate, forming Metaphen sodium.

1:2,500 disinfecting solution, List No. 3543, 1 part metaphen dissolved in 2,500 parts of an aqueous solution containing 0.81 Gm. sodium hydroxide U.S.P., 12.5 Gm. sodium carbonate, ethylene glycol 500 Gm., and benzyl alcohol 100 Gm.

Saponated cresol solution U.S.P.

Lilly's cresol solution U.S.P., Solution 7, containing water not over 15 per cent, glycerin not over 4 per cent.

Benzalkonium chloride U.S.P. (Zephiran, Winthrop).

A mixture of alkyl dimethyl-benzylammonium chlorides of the general formula, $C_6H_5CH_2N(CH_3)_2RCl$, in which R represents a mixture of alkyls from $C_8H_{17}$ to $C_{18}H_{37}$. 1:1,000 solution.

Sodium ethyl mercuri thiosalicylate, $C_2H_5HgSC_6H_4COONa$ (Merthiolate or Merthiolate Sodium, N.N.R., 1947, Lilly).

1:1,000 solution, containing 1 Gm. merthiolate in 1,000 c.c. of water buffered with 1.4 Gm. of sodium borate and containing sodium chloride to make the solution approximately isotonic.

Isopropyl alcohol 99 per cent, (Merck)

Hydrogen peroxide U.S.P. (3 per cent hydrogen peroxide)

Formaldehyde solution U.S.P. (37 per cent formaldehyde). Used in the preparation of a 3 per cent solution, with additions described later.

Amphyl (Lehn and Fink)

"Contains chloroxylenol, butyl-, amyl-, and phenyl phenol isomers and soap, isopropyl alcohol 20 per cent by volume, propylene glycol and glycerol not more than 20 per cent."

Instructions for use: "Prepare 2 per cent by volume in 100 parts water containing 10 parts alcohol and 5 parts glycerine."

Benzyl alcohol (Merck)

Phenol (Merck)

The following preparations were obtained directly from the firms manufacturing them:

Lauryl dimethylbezylammonium chloride, crystals ("DC 12," Stillman Company)

Benzalkonium chloride U.S.P. (Zephiran, Winthrop). 12.8 per cent concentrate.

Nitromersol N. F. (Metaphen, Abbott). Proportions as for 1:2,500 solution List No. 3503, previously described, but with 1 Gm. Metaphen.

Nitromersol N. F. (Metaphen, Abbott). Proportions as for 1:2,500 Metaphen disinfecting solution List No. 3543, previously described, but containing 1 per cent Metaphen.

Ethyl alcohol 70 per cent by volume and 70 per cent by weight were prepared from absolute alcohol. Benzyl alcohol 4 per cent by weight was prepared from pure benzyl alcohol. Aqueous iodine was made by dissolving 20 Gm. of iodine crystals in distilled water with the aid of 40 Gm. of potassium iodide and making the solution to 900-950 c.c. The iodine content was determined by means of tenth normal sodium thiosulfate and the solution diluted to exactly 2 per cent.

The formaldehyde solution was prepared according to the directions of Tainter, Throndson, Beard, and Wheatlake,[17] except that the small amounts of essential oils used were slightly different, oil of lavender instead of oil of geranium, and cassia instead of cinnamon. The ingredients of this solution as given by Tainter, Throndson, Beard, and Wheatlake[17] are as follows (they are mixed in the order given):

| | |
|---|---|
| Isopropyl alcohol | 1,000 c.c. |
| Oil of rose geranium | 2 c.c. |
| Oil of cinnamon or cassia | 4 c.c. |
| Water | 780 c.c. |
| Sodium nitrite | 4 Gm. |
| Mono- (or tri-) ethanolamine | 60 c.c. |
| Formaldehyde solution U.S.P. | 160 c.c. |

The amount of formaldehyde is approximately 3 per cent, since there are 8 parts of the formaldehyde solution in each 100 parts of the final solution.

A 5 per cent stock solution of phenol was made by dissolving in water somewhat more than the amount needed, determining the phenol content by the standard assay method (with N/10 bromine and N/10 sodium thiosulfate) and diluting the solution exactly to 5 per cent. The dilutions needed were made from this stock solution by careful measurement with bulb pipettes. Orthophenyl phenol was brought into solution by the aid of coconut oil soap and alkali, according to the directions of Tilley, MacDonald, and Schaffer.[18] Phenolor, which is a 13 per cent solution, was diluted with distilled water to 2 per cent; in one instance a 4 per cent solution was used. The concentrated solution is best added in small amounts to the water in order to obtain a clear solution. Saponated cresol was prepared in dilutions by volume, "5 per cent saponated cresol" indicating 5 parts of the concentrate, as purchased, made up to 100 parts with distilled water. The "2 per cent" and "3 per cent" solutions similarly indicate 2 parts and 3 parts of the concentrate in 100 parts of water. The solutions of Zephiran, except for one lot of 1:1,000 which was tested as purchased, were made from the concentrated 12.8 per cent solution, "1 per cent" indicating 1 part in 100 parts of water.

"DC 12" is very soluble in water, and the desired solutions were made by weighing the crystals and adding water to give the required concentration.

Sterile pipettes and containers were used in handling the chemical solutions in order to avoid the possibility of introducing bacterial spores which are not killed by disinfectants except after prolonged periods of exposure.

The formaldehyde solution[17] and the "DC 12" solutions were found to be very stable, giving results after standing many months which were identical with those obtained with the fresh solutions.

*Toxicity of chemicals used.*—None of the chemicals described in the foregoing paragraphs proved toxic for mice in the small amounts remaining on the tail. Toxicity may be a factor in tests of this sort, however, as shown by Spaulding and Bondi,[19] and as we ourselves found in attempting to evaluate an ammoniacal solution of silver nitrate. In the guinea pig experiments we encountered the problem of toxicity in the case of formaldehyde solution, iodine, and the quaternary ammonium compound "DC 12" but were able to overcome it as described later on.

*Antirusting agents.*—When an antirusting agent was required, as in the case of "DC 12" and Phenolor, 0.2 per cent sodium nitrite was used. The antirust tablets available commercially would no doubt be equally satisfactory.

## RESULTS

It should be noted that the tests reported for a given disinfectant were made at different times in small groups of animals. In the mouse tests 10 to 15 mice were tested at one time against each of 2 or 3 disinfectants, together with at least 3 control mice in which the disinfectant was replaced with physiological salt solution. We found that the results of any one such test were readily reproducible in succeeding tests of the same size, with one exception, noted later on. The results recorded in the charts are based on repeated tests, and the number of mice used for testing any one disinfectant varied between 36 and 50.* We obtained the mice from a single source and in lots of 100 to 200 at intervals of approximately 2 weeks. The plan as outlined enabled us to compare several disinfectants in a given lot of mice. It seemed to us that this procedure was preferable to testing 2 or 3 mice with each of several disinfectants on a given day.

*Iodine.*—Although we were chiefly interested in disinfection of instruments, we tested iodine as a sort of "positive control," since Nungester and Kempf[12] had reported only occasional instances in which it failed to kill both the pneumococcus and the streptococcus, even in 2 per cent concentration and in aqueous (potassium iodide) solution. It will be noted by comparison of Figs. 1, 2, and 4 that 2 per cent aqueous iodine was bactericidal to all 3 bacteria against which it was tested. Only 2 of the pneumococcus series (58 mice) and one of the streptococcus series (42 mice) died of infection, and none of the 12 guinea pigs receiving tubercle bacilli exposed to iodine became infected, although all the saline controls inoculated at the same time developed heavy infections.

It may be noted here that Emmons[20] has reported that iodine is fungicidal. Perhaps iodine is less selective than other disinfectants.

*When the mortality in a group of 20 to 30 mice was approximately 100 per cent, the disinfectant in question usually was not tested further.

*Saponated cresol U.S.P.*—This solution was also effective against all 3 bacteria, but while a solution of 5 parts in 100 was required to kill the pneumococcus and streptococcus, one of 2 parts in 100 was adequate for the tubercle bacillus. Saponated cresol has long been the disinfectant of choice among bacteriologists. Unfortunately it has a marked odor, unpleasant to many persons, which is regarded as sufficient to exclude its use in dental offices.

*Formaldehyde solution* (Tainter, Throndson, Beard, and Wheatlake[17]).—This solution, which contains approximately 3 per cent formaldehyde, has a pleasant odor. It proved to be effective not only against the pneumococcus and the streptococcus but also against the tubercle bacillus, if we may accept the results in the 6 animals which received tubercle bacilli exposed for at least 10 minutes to this solution. All the controls receiving the same culture in saline became heavily infected with tubercle bacilli.

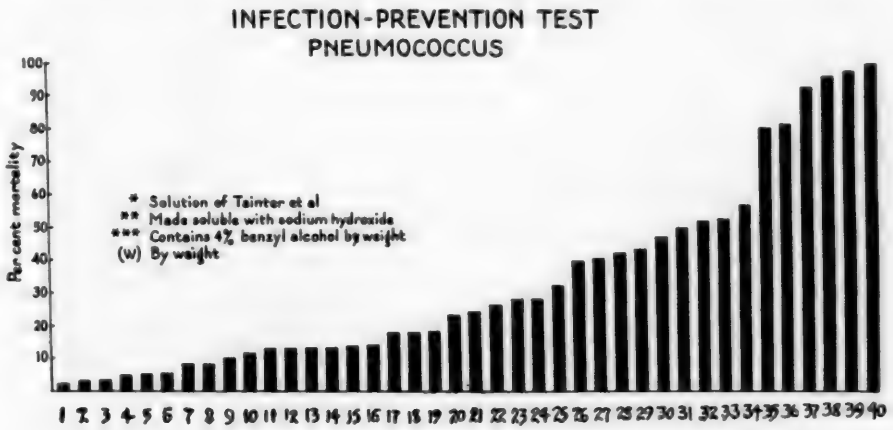
INFECTION-PREVENTION TEST
PNEUMOCOCCUS
Per cent mortality
100
90
80
70
60
50
40
30
20
10
* Solution of Tainter et al
** Made soluble with sodium hydroxide
*** Contains 4% benzyl alcohol by weight
(W) By weight
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40

Fig. 1.

1. Orthophenyl phenol 2 per cent + coconut oil soap 4 per cent
2. Iodine 2 per cent
3. "DC 12" 1 per cent
4. Formaldehyde 3 per cent*
5. Zephiran 1 per cent
6. Phenolor 2 per cent
7. "DC 12" 1 per cent tincture
8. Saponated cresol 5 per cent
9. Phenol 2 per cent
10. Metaphen 1 per cent**
11. "DC 12" 0.3 per cent
12. Formaldehyde 1 per cent*
13. Ethyl alcohol 70 per cent (W)
14. Saponated cresol 3 per cent
15. "DC 12" 0.25 per cent
16. Isopropyl alcohol
17. Ethyl alcohol 70 per cent (V)
18. "DC 12" 0.5 per cent
19. Ethyl alcohol 70 per cent (W) Benzyl alcohol 4 per cent (W)
20. Metaphen 0.04 per cent***
21. Phenolor 1 per cent
22. Metaphen 1 per cent***
23. Saponated cresol 4 per cent
24. Orthophenyl phenol 1 per cent + coconut oil soap 2 per cent
25. Amphyl, etc. 2 per cent
26. Saponated cresol 1 per cent
27. Benzyl alcohol 4 per cent (W)
28. Zephiran 0.1 per cent from 12.8 per cent
29. "DC 12" 0.1 per cent
30. Phenol 1 per cent
31. Formaldehyde 0.6 per cent*
32. Saponated cresol 0.5 per cent
33. "DC 12" 0.2 per cent
34. Orthophenyl phenol 0.5 per cent + coconut oil coap 1 per cent
35. Zephiran 0.1 per cent
36. Metaphen 0.1 per cent
37. Hydrogen peroxide 3 per cent
38. Metaphen 0.04 per cent**
39. Merthiolate 0.1 per cent
40. Physiological salt solution

W = weight; V = by volume.

Isopropyl alcohol, which is present in this solution, has considerable bactericidal action of its own (Fig. 3). It should be noted that the formaldehyde

solution had to be used in full strength, as prepared, to be effective even in the case of the pneumococcus, which usually proved the easiest of the three bacteria to kill (Fig. 1).

*Phenol.*—Phenol was active against the tubercle bacillus in 1 per cent solution (Fig. 4) and against the pneumococcus in 2 per cent solution (Fig. 1), but 40 per cent of the mice receiving streptococci exposed to 2 per cent phenol died, and a 4 per cent concentration was also not satisfactory for use against the streptococcus (Fig. 2).

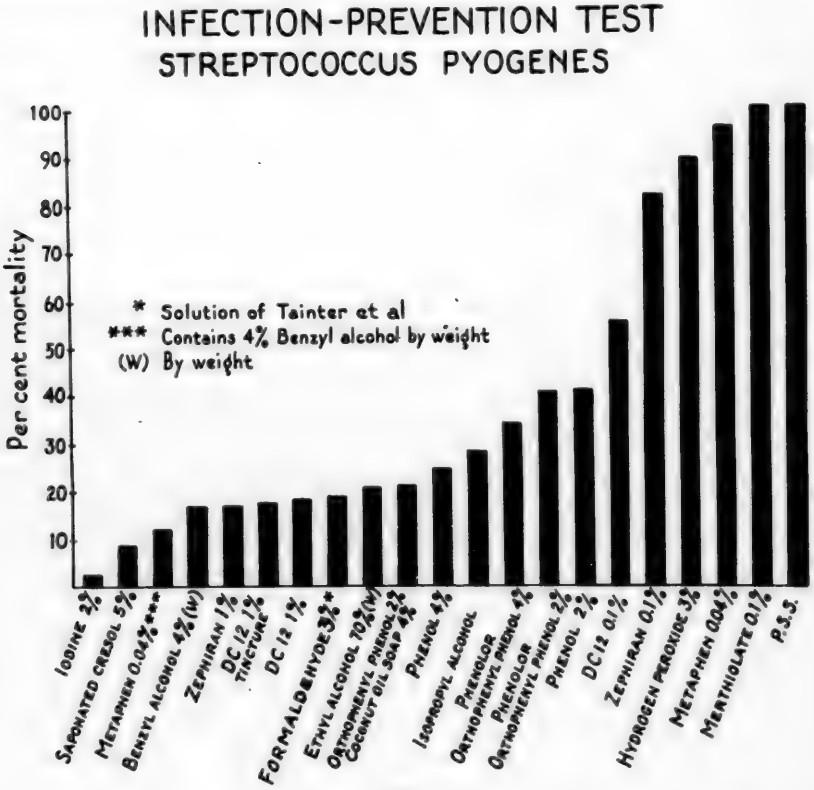


Fig. 2.

*Orthophenyl phenol.*—Tilley, MacDonald, and Schaffer,[18] in 1931, recommended a solution of orthophenyl phenol, dissolved in coconut oil soap, which they had found to be bactericidal for *Myco. tuberculosis.* They reported a mortality of 2 per cent in guinea pigs receiving infected spleen in an equal volume of the disinfectant. In mice receiving streptococci exposed to the solution, the mortality was 21 per cent. We have verified these findings in our experiments, which show that either 2 per cent orthophenyl phenol or the commercial preparation Phenolor kills *Myco. tuberculosis* in 10 minutes (Fig. 4) but is not very active against the hemolytic streptococcus.

In connection with the infection-prevention tests of Phenolor against the hemolytic streptococcus, we encountered our first and only failure to obtain comparable results in repeated tests of a disinfectant. In several groups of mice the mortality varied from a low of 19 per cent to a high of 74.4 per cent. The mortality shown in Fig. 2, 41 per cent, is the average for a total of 104 mice.

We cannot account to our satisfaction for these discrepancies. We had noticed the formation of considerable precipitate when we used 2 per cent Phenolor in place of saponated cresol in jars for discarded pipettes. There was also some precipitate in a bottle of concentrated stock solution after several weeks. When we compared material from the old stock bottle with that from a fresh bottle, using 20 mice in each experiment, we found a marked difference in effectiveness. The mortality was 30 per cent for the group of mice for which the old material was used, as compared with 5 per cent for the group tested with the fresh material. It may be, therefore, that the irregular results were due in part to instability of the solution. We had no difficulty with precipitation in our laboratory preparation of orthophenyl phenol, which was used in 28 mice tested with streptococcus, of which 6 succumbed (21.4 per cent).

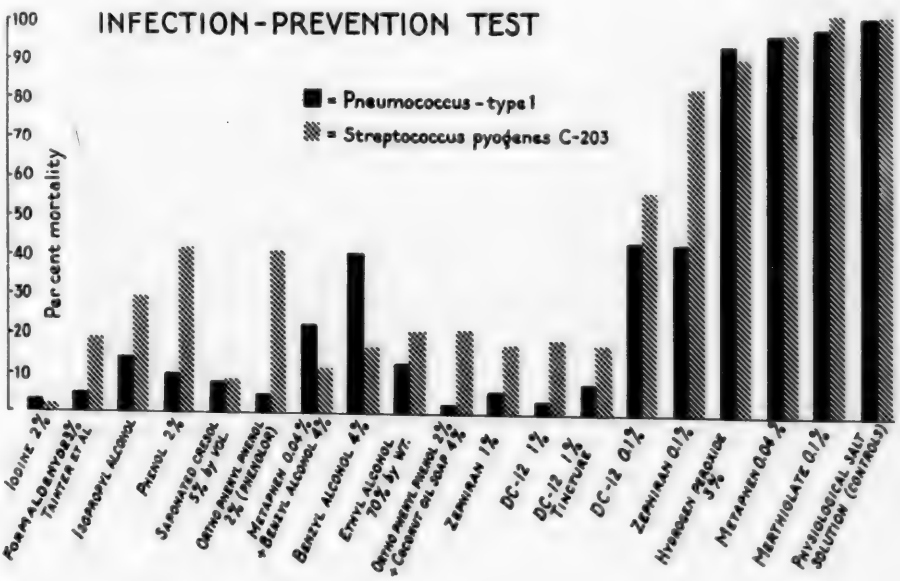


Fig. 3.

The pneumococcus was readily killed, either by our laboratory preparation of orthophenyl phenol, or by 2 per cent Phenolor. The mortality from pneumococcus infection was 2.4 per cent for our preparation (tests in 41 mice) and 5.7 per cent for 2 per cent Phenolor (tests in 35 mice).

There were no infections in guinea pigs, 20 in all, which received tubercle bacilli previously exposed either to our laboratory preparation of orthophenyl

phenol or to 2 per cent Phenolor. All control animals, receiving the same culture suspension exposed only to physiological saline solution, became heavily infected.

There is a possibility, when animal experiments are made at different seasons of the year, that seasonal conditions may account for irregularities. Seasonal irregularities were reported by Brown and Pearce[21] in their work on syphilis, and Friedemann[22] has reported seasonal variations in the metabolism of the pneumococcus.

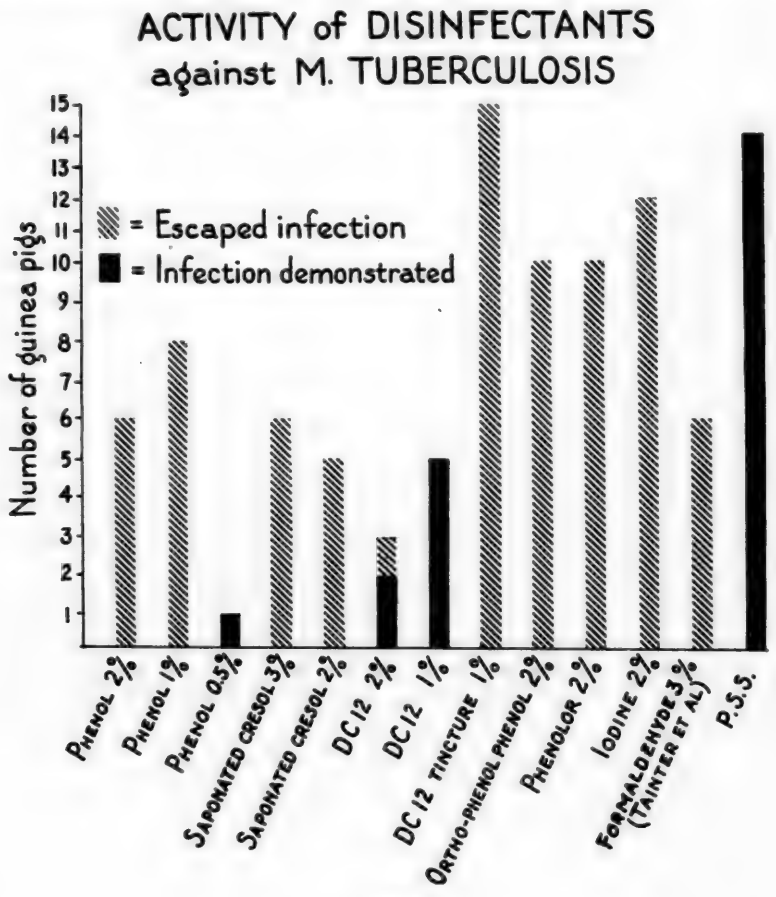


Fig. 4.

Orthophenyl phenol has the great advantage over saponated cresol of being practically odorless.

*Mercurials.*—As examples of the organic compounds of mercury, we tested Merthiolate and Metaphen against the pneumococcus and the streptococcus. The labels on the bottles of Metaphen Disinfecting Solution specifically except the tubercle bacillus, as well as bacterial spores, from the range of activity of this solution.

Our results with Merthiolate confirmed those of Nungester and Kempf,[12] showing that 0.1 per cent Merthiolate was entirely ineffective in killing either the pneumococcus or the streptococcus. Aqueous Metaphen, whether in 1:1,000 or 1:2,500 dilution, was likewise useless against both these organisms, but even the more dilute solution was rendered a good disinfectant especially for the streptococcus when benzyl alcohol was added in a concentration of 4 per cent

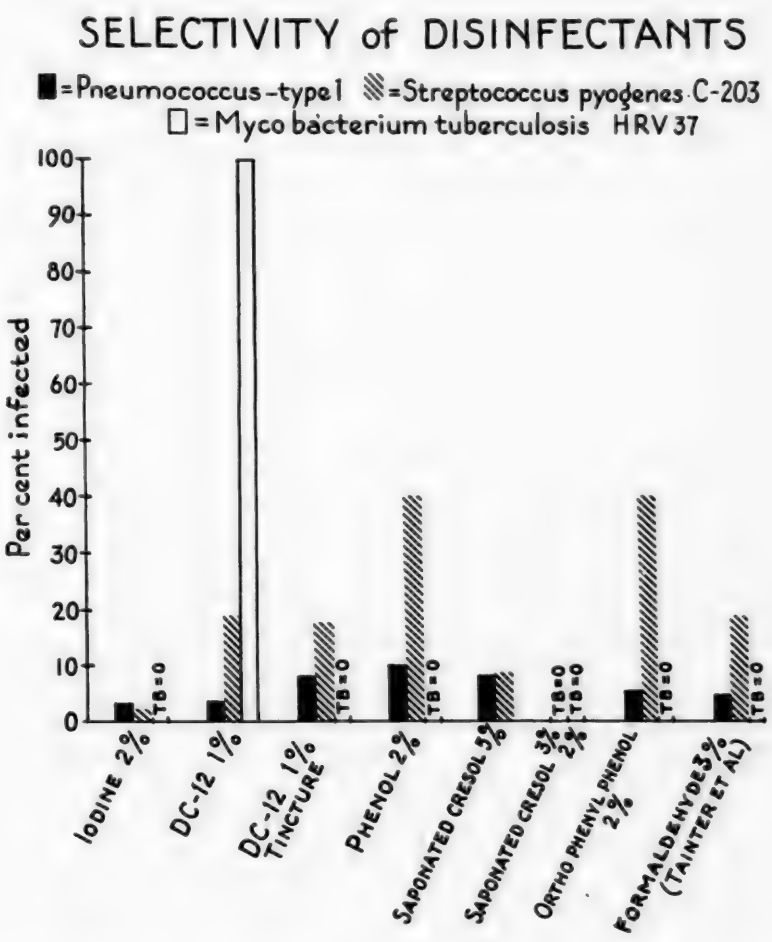


Fig. 5.

by weight (Fig. 2). The addition of benzyl alcohol did not make as much difference in the potency of Metaphen against the pneumococcus, a fact which is readily explained by comparing the action of benzyl alcohol alone with that of Metaphen and benzyl alcohol on the two bacteria (Fig. 3). In all other instances the pneumococcus was more readily killed than the streptococcus, but in the case of benzyl alcohol and of Metaphen containing it, the reverse is true.

Concentrated solutions of Metaphen (1 per cent) were much more active against the pneumococcus than either 1:2,500 or 1:1,000 dilutions, irrespective of whether they contained benzyl alcohol or not. The idea that mercurials were satisfactory disinfectants in very high dilution arose from tests in vitro, in which their ability to inhibit multiplication in minute amounts resulted in confusion of bacteriostatic with bactericidal action. It would seem from the experiments which are related in the foregoing paragraph that certain mercurials may be bactericidal as well as bacteriostatic if used in sufficient concentration.

*Alcohols.*—Ethyl and isopropyl alcohols were almost equally bactericidal to both streptococcus and pneumococcus, very slightly more so for the pneumococcus. There was no appreciable difference in effectiveness of ethyl alcohol in favor of the dilution prepared by weight rather than by volume (Fig. 1). The addition of 4 per cent benzyl alcohol to ethyl alcohol did not increase its killing power. The effectiveness of benzyl alcohol versus the hemolytic streptococcus has already been discussed in connection with its use in Metaphen disinfecting solution.

Comparison of our results with those of Nungester and Kempf shows that ours were very much more favorable with respect to ethyl alcohol (Fig. 3). The effectiveness of alcohol as a disinfectant appears to be difficult to assess, since the literature contains many reports which are at variance with one another.

*Quaternary ammonium compounds.*—Lauryl-dimethylbenzylammonium chloride ("DC 12") is a pure crystalline compound which is extremely soluble in water, has a faint pleasant odor, and is nonirritating to the skin. A 1 per cent solution is almost as effective against the pneumococcus as 2 per cent iodine. It is less bactericidal for the streptococcus, whether made up in water or in a 1 per cent tincture, the mortality in mice being 21 or 18 per cent, respectively, for the 2 solutions. A 1:1,000 solution was much less useful, nearly one-half of the experimental mice dying when the disinfectant was used in this dilution, even when the pneumococcus was the test organism.

Zephiran behaved much like "DC 12," being effective against the pneumococcus in 1 per cent solution, less effective against the streptococcus, and only 50 per cent active against either in a 0.1 per cent solution.

"DC 12" was tested also against *Myco. tuberculosis*, both in relatively concentrated aqueous solutions and in 1 per cent tincture. Although the aqueous solution was unable to kill the tubercle bacillus, the tincture in 1 per cent concentration proved highly satisfactory for this purpose, all 15 guinea pigs escaping infection when the organisms were exposed to "DC 12" tincture before injection, while controls in which salt solution was substituted for the disinfectant all became heavily infected. "DC 12" was toxic to guinea pigs on intraperitoneal injection, but it was readily detoxified with 1 per cent soap solution after the tubercle bacilli had been exposed to it but before the mixture was inoculated.

A third quaternary ammonium compound tested against the hemolytic streptococcus was cetyldimethylethylammonium bromide (Radiol), which was

furnished in a "germicidal solution concentrate." It was diluted 1:3 with water, as indicated in the directions. The mortality among the mice was very high in this experiment, 18 of 21 mice succumbing to infection.

*Hydrogen peroxide U.S.P.*—It was a surprise to us to find that 3 per cent hydrogen peroxide* had no bactericidal action against either of the two pyogenic cocci studied.

*Amphyl.*—Amphyl disinfecting solution showed relatively low effectiveness against the pneumococcus (Fig. 1) and was not tested against the streptococcus.

## DISCUSSION AND SUMMARY

The results for each microorganism are recorded in Figs. 1, 2, and 4. In Fig. 3 a comparison is made of the killing power of the various disinfectants for the pneumococcus and the streptococcus, and in Fig. 5 the percentage of fatal infections in mouse tests with the two pyogenic cocci is shown in parallel with the percentage of infections in guinea pig tests with *Myco. tuberculosis.*

It is evident that there are striking differences in the effectiveness of the various disinfectants according to which bacterium was used as the test organism. Iodine and saponated cresol were almost completely effective against all three of the bacteria used in the tests. The 3 per cent formaldehyde solution of Tainter, Throndson, Beard, and Wheatlake, and the 1 per cent tincture of "DC 12" killed both the pneumococcus and the tubercle bacillus and were reasonably active against the hemolytic streptococcus. Zephiran behaved much like "DC 12" against the pyogenic cocci. It is to be noted that it was necessary to use these two quaternary compounds in relatively high concentration (1 per cent), also that compounds of this type are not necessarily bactericidal. For example, cetylmethylethylammonium bromide (Radiol) appeared to be inactive when used in the concentration recommended by the makers. Phenol and the phenol derivative, orthophenyl phenol (Phenolor) failed in the case of the streptococcus, though active against both the tubercle bacillus and the pneumococcus. Aqueous "DC 12" was useless against the tubercle bacillus, even in 1 per cent solution; the fact that the tincture killed this organism is especially interesting in view of the work of Smith,[23] which indicated that ethyl alcohol is bactericidal for the tubercle bacillus. Although the tubercle bacillus is usually thought of as harder to kill than the streptococci or pneumococci, because of its resistance to acids, alkalies, chlorine, and mercurials, it is noteworthy that in the case of saponated cresol a solution of 2 parts in 100 was sufficient to kill the tubercle bacillus, while 5 parts in 100 was necessary for the two pyogenic cocci.

On the basis of the comparisons shown in Figs. 3 and 5, it would seem that a safe choice for disinfection of dental instruments would be 5 per cent saponated cresol or the 3 per cent formaldehyde solution of Tainter, Throndson, Beard, and Wheatlake. A 1 per cent tincture of "DC 12" would be nearly as good, though not quite so effective against the hemolytic streptococcus, while Metaphen Disinfecting Solution, which contains benzyl alcohol, would be highly

*The tests were made with hydrogen peroxide which was taken from a previously unopened bottle and used immediately to prevent spontaneous decomposition.

satisfactory in the case of the hemolytic streptococcus. Aqueous solutions of Metaphen and Merthiolate should certainly not be relied upon, and orthophenyl phenol would seem to be of doubtful value, in view of its lack of ability to kill the hemolytic streptococcus, even though it is very effective against the other two bacteria, especially the tubercle bacillus.

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# PHYSIOLOGICAL TOOTH MIGRATION AND ITS SIGNIFICANCE FOR THE DEVELOPMENT OF OCCLUSION

## I. The Biogenetic Course of the Deciduous Dentition

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### INTRODUCTION

ANY attempt to develop preventative methods in the field of orthodontics must be based on adequate knowledge of normal growth and development of the dentitions and the many modifying factors. A review of the literature reveals little authentic information on which the present-day concept of the development of occlusion is based.

Delabarre, in 1819, described for the first time the spacing of anterior deciduous teeth between the ages of 4 and 6 years and suggested that the purpose was to make allowance for the permanent ones.[15] This concept prevails among most writers of recent textbooks (Dewey-Anderson[1, 6] Korkhaus,[14] McCoy,[20] Salzmann,[26] Schour and Massler,[27] and Strang.[35]

Zsigmondy[38] in 1890, was the first to measure the lengths of the dental arches of three series of models of the same individual between 6 and 17 years of age. After Angle[30] published his *Malocclusion of the Teeth* in 1907, the main interest turned from genetics to the systematics of malocclusion. Thus, Angle's theory of the fixed position of the upper first molars became an established law in orthodontics.

In 1910, Zielinsky[37] concluded from measurements of four skulls and some models of various individuals that, between the ages of 4 and 6 years, the upper anterior deciduous teeth move forward and laterally. This spreading of the upper dental arch would cause a spacing of the deciduous anteriors and simultaneously a forward migration of the mandibular deciduous teeth into the enlarged maxillary arch. This concept is known as the so-called "physiological mesial shift" of the mandibular teeth.[1, 14, 20, 26, 35]

In 1922, Franke,[7] from numerous skull measurements, calculated a shortening of 3.7 mm. in the lower dental arch following the shedding of the deciduous teeth. Simon,[34] in 1924, presented his biometric law of the "orbit-cuspid relationship" establishing the possibility of positional changes of the upper permanent molars. Kantorowicz[13] was the first to emphasize the necessity for a genetic approach to the classification of dentofacial and occlusal anomalies on the basis of a large number of serial models of development. In 1927 Schwarz[28] reported a comprehensive anatomical study of the problem. He accounted for occlusal adjustment by means of tooth morphology. Friel's[8] de-

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scription of the development of occlusion represented a synopsis of all the contemporary studies. In 1929 Lewis[17] measured the changes in width of the dental arches during the periods of the deciduous and mixed dentitions on a large number of serial models. He came to the conclusion that these measurements offered no solution in diagnosis of developing malocclusion. In 1931, Korkhaus and Neumann[15] reported similar measurements of "Growth of the Jaws During Eruption of the Permanent Incisors" based on a survey of 35 individuals with normal development thus laying the foundation for the biogenetic concept of orthodontics. The investigations by Goldstein and Stanton[10, 11] dealt with the same problem of arch development and the incisal overbite. The studies of Cohen,[5] in 1940, had to do with the changes of the intercanine distances in the deciduous and succeeding dentitions separately in boys and girls. Clinch[4] and Sillman[32, 33] were the first to present developmental series of dentitions from birth.

The histologic contributions of Brash,[3] Reichborn,[25] Meyer and Churchill,[21] Noyes and Schour,[23] Orban,[24] and Weinmann and Sicher[31, 36] have clarified much in the problems of growth and development of the dentition.

For the purpose of ascertaining more detailed information on the physiological changes in the human dental arches, a clinical study of their growth and development in 60 children was made over a period of 8 years. Through careful recording an attempt was made to determine the biogenetic course of the deciduous dentition as well as the biogenesis of the accessional and successional teeth.*

## I. The Biogenetic Course of the Deciduous Dentition

In order to determine developmental changes in the deciduous dental arches, plaster reproductions were made annually of 30 children; 29 of these had their first impressions taken between 3 and 4½ years of age and one at birth. A fine caliper was used on each cast to make the following measurements:

(a) The length in mm. of the deciduous arch, i.e., the perpendicular distance from a line connecting the two postlactea (pl), to the infradentale (id) or prosthion (pr) (Figs. 1*A*, 2*A*).

(b) The width in mm. between the deciduous canines, i.e., intercanine distance (c-c). This was measured between the centers of the lingual cinguli at the gingival margin instead of between the cusp points, which flatten out as attrition progresses. The possible error by this procedure does not exceed ±0.2 mm. (Fig. 2*B*).

(c) The width in mm. between the two second deciduous molars, i.e., intermolar distance ($m_2$-$m_2$). This measurement was taken between the lingual developmental grooves at the gingival margin instead of between the changing occlusal surfaces (Fig. 1*B*).

### FINDINGS

Data of the above measurements of each case are presented in Table I for a minimum of two different ages.

*These topics will be presented in four papers. The second paper deals with "The Biogenesis of Accessional Dentition;" the third with "The Biogenesis of Successional Dentition," and the fourth paper with "The Biogenesis of Overbite."

## Table I

### Showing Length and Width of Upper and Lower Dental Arches in mm. During the Period of the Deciduous Dentition

| Case | Age (Years) | A Length of Arch | | | B Width of Maxilla | | | C Width of Mandible | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Max. | Mand. | | C-c | $M_2$-$m_2$ | Spaces | C-c | $M_2$-$m_2$ | Spaces |
| 1 | 4-5 | 27 | 26 | | 22.5 | 30.5 | | 18 | 28 | |
| | 5-6 | 27 | 25 | a | 22.5 | 30.5 | small | 18 | 28 | no |
| 2 | 4-5 | 26 | 23 | | 21.5 | 30.5 | | 18 | 28 | |
| | 5-6 | 26 | 22.5 | a | 21.5 | 30.5 | small | 18 | 28 | no |
| 3 | 4-5 | 25 | 22 | | 22 | 29.5 | | 16 | 28 | |
| | 5-6 | 25 | 22 | | 22 | 30 | + | 16 | 28 | + |
| 4 | 4-5 | 25 | 22.5 | | 25 | 30 | | 18 | 28 | |
| | 5-6 | 25 | 21.5 | | 25 | 30 | + | 18 | 28 | + |
| 5 | 4-5 | 25 | 22.5 | | 20 | 26 | | 17.5 | 24.5 | |
| | 5-6 | 25 | — | b | 20.5 | 26.5 | no | 18 | 25 | no |
| 6 | 4-5 | 27 | 24.5 | | 25.5 | 27 | | 18 | 27 | |
| | 5-6 | 26 | 24.5 | a | 25.5 | 27 | no | 18 | 27 | no |
| 7 | 4-5 | 26 | 24 | | 23.5 | 31 | | 17.5 | 27 | |
| | 5-6 | 26 | 24 | | 24 | 31 | + | 17.5 | 27 | + |
| 8 | 4-5 | 26 | 24.5 | | 24.5 | 29.5 | | 18 | 26.5 | |
| | 5-6 | 26 | 24.5 | | 24.5 | 29.5 | + | 18 | 26.5 | + |
| 9 | 4-5 | 26 | 23 | | 23 | 31 | | 18 | 28 | |
| | 5-6 | 26 | 23 | | 23 | 31 | + | 18 | 28 | + |
| 10 | 4-5 | 28.5 | 23 | | 24.5 | 32 | | 18 | 29 | |
| | 5-6 | 28.5 | 23 | | 24.5 | 32 | + | 18 | 29 | + |
| 12 | 4-5 | 27.5 | — | | 22 | 29 | | 18 | — | |
| | 5-6 | 27.5 | — | | 22 | 29 | | 18 | — | + |
| 13 | 4-5 | 25 | 22.5 | | 23 | 29 | | 19 | 26.5 * | |
| | 5-6 | 25 | 22.5 | b | 23 | 29 | + | 19 | 27 | + |
| 16 | 5-6 | 29 | 25 | | 24 | 34 | | 20 | 30.5 | |
| | 6-7 | 29 | 25 | b | 24.5 | 34.5 | + | 20 | 31 | + |
| 17 | 5-6 | — | 24 | | 21 | 31 | | 20 | 29 | |
| | 6-7 | — | 23 | b | 21 | 31 | no | 20 | 29 | no |
| 18 | 5-6 | 25 | 21 | | 19 | 27.5 | | 17 | 27 | |
| | 6-7 | 24 | 21 | b | 20 | 27.5 | + | 17.5 | 27 | + |
| 19 | 5-6 | | | | 22.5 | 26 | | 17 | 25 | |
| | 6-7 | | | | 22.5 | 26 | no | 17 | 25.5 | no |
| 24 | 5-6 | 28 | 25 | | 26 | 30.5 | | 18 | 30 | |
| | 6-7 | 28 | 25 | | 26 | 30.5 | no | 18 | 30 | no |
| 26 | 4-5 | 25 | 23 | | 17.5 | 27 | | 18 | 28.5 | |
| | 5-6 | 25 | 23 | | 17.5 | 27 | no | 18 | 28.5 | no |
| 27 | 4-5 | 26 | 24 | | 23 | 29 | | 19 | 26 | |
| | 5-6 | 26 | 24 | | 23.5 | 29.5 | + | 19 | 26 | + |
| 31 | 4-5 | 26 | 23 | | 20 | 29 | | 16 | 27 | |
| | 5-6 | 26 | 23 | | 20 | 29 | no | 16 | 27 | no |
| 32 | 3-4 | 25 | 23 | | 21 | 28 | | 17 | 26 | |
| | 5-6 | 25 | 23 | | 21 | 28 | small | 17 | 26 | small |
| 33 | 5-6 | 25.3 | 23 | | 23 | 30 | | 18 | 27 | |
| | 6-7 | 25.3 | 23 | | 23 | 30 | + | 18 | 27 | + |
| 40 | 4-5 | 29 | 24.5 | | 21 | 30 | | 17 | 26 | |
| | 5-6 | 29 | 24.5 | | 21 | 30 | no | 17 | 26 | no |
| 42 | 3-4 | 27.5 | 23.5 | | 21.5 | *25 | | 16.5 | *25.5 | |
| | 5-6 | 26.5 | 23 | * | 21.5 | 26.5 | small | 16 | 26 | no |
| 43 | 3-4 | — | 26 | * | *20.5 | 28 | | 19 | 28.5 | |
| | 5-6 | — | 25 | | 21 | 28 | + | 19 | 28.5 | + |
| 48 | 4-5 | 26 | 24.5 | | 23.5 | 31 | | 21 | 31 | |
| | 5-6 | 26 | 24.5 | | 23.5 | 31 | + | 21 | 31 | + |
| 50 | 4-5 | 25 | 22 | | 22 | 33 | | 20 | 32 | |
| | 6-7 | 25 | 22 | | 22 | 33 | + | 20 | 32 | + |
| 57 | 3-4 | (27) | 24 | | *23.5 | *— | | *18 | 29 | |
| | 4-5 | 26 | 23 | * | 24.5 | 33 | | 19.5 | 29 | |
| | 5-6 | 26 | 23 | | 24.5 | 33 | + | 19.5 | 29 | + |
| 58 | 3-4 | 31 | 26 | | 23 | 30.5 | | 19 | 28 | |
| | 4-5 | 30 | 26 | * | *23.5 | 30.5 | + | 19 | 28 | + |
| | 5-6 | 30 | 26 | | 23.5 | 30.5 | | 19 | 28 | |
| 59 | 3-4 | 28 | 24.5 | | 21 | 27 | | 17 | 25.5 | |
| | 4-5 | 27 | 24.5 | | 21 | 27 | no | 17 | 25 | small |
| | 5-6 | 27 | (24) | 1-1 | 21 | 27 | | (18) | 25 | 1-1 |

* = deciduous canine or second deciduous molar erupting.
a = interdental caries or restoration.
b = beginning eruption of lower first permanent molar.
\+ = with interdental spaces, Type I.
no = without spacing, Type II.

Table I, Column A, reveals that during the period from 4 to 6 years the length of the maxillary dental arches remains unchanged in 25 cases (89 per cent) and that of the mandibular arches is constant in 23 cases (83 per cent). A slight decrease in length is noted in 6 cases in the maxilla and in 8 cases in the mandible, mainly between 3 and 4 years of age. In only 3 cases is this shortening coincidental for both arches. The decrement ranges between 0.5 mm. and 1.5 mm. No increase in length is observed in any of these cases.

Table I, Column B, shows the measurements of the transverse width of the maxillary deciduous arches at different periods. No change in the intercanine width between 4 and 6 years is observed in 24 (80 per cent) of the cases. Five cases reveal an increase in width of 0.5 mm. and one case of 1 mm. during the same interval. The distance between the second deciduous molars is not altered in 25 (83 per cent) of the cases. In 4 cases an increment of 0.5 mm. and in one case of 1 mm. is noted. Twenty-one cases (70 per cent) show interdental spaces between the upper anteriors, while 9 cases (30 per cent), exhibit no spaces.

Table I, Column C, gives the corresponding data for the mandibular deciduous dental arches. The lower intercanine width remains equal in 26 cases (86 per cent and similarly for the intermolar distance in 24 cases (80 per cent). An increment in intercanine width is found in 3 cases, and a decrement in one case. Nineteen cases (63 per cent) show spacing in the lower anteriors and 11 cases (37 per cent) no spaces.

TABLE II

SHOWING THE AVERAGE INTERCANINE WIDTH IN DECIDUOUS DENTITIONS OF TYPE I WITH INTERDENTAL SPACES AND TYPE II WITHOUT INTERDENTAL SPACES

| | MANDIBULAR ARCH | | | MAXILLARY ARCH | | |
|---|---|---|---|---|---|---|
| TYPE | NO. CASES | MINIMUM-MAXIMUM VARIATION | AVERAGE WIDTH | NO. CASES | MINIMUM-MAXIMUM VARIATION | AVERAGE WIDTH |
| I | 28 | 16/21 | 18.9 | 32 | 19 /26 | 23.2 |
| II | 20 | 16/20 | 17.4 | 16 | 17.5/25 | 21.5 |
| Difference between Type I and II: 1.5 mm. | | | | | | 1.7 mm. |

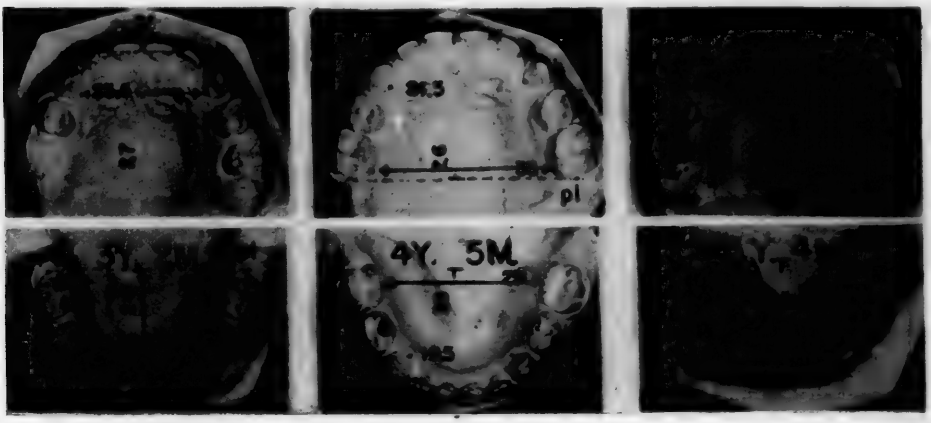
Table II presents the average intercanine width at the age of 5½ years noted separately for the cases with interdental spaces (Type I) and without interdental spaces (Type II). It shows that in maxillary arches with spaces the average value is 1.7 mm. higher than in arches without spaces. A similar difference of 1.5 mm. is seen in the mandibular arches.

The comparison of the plaster reproductions of each case taken seriatim at various ages revealed conclusions in the following respects:

1. *The Length of the Deciduous Arches.*—From about the age of 4 years until the eruption of the permanent molars the sagittal dimensions of upper and lower dental arches remained unchanged. A slight decrease in this dimension can occur either as the result of mesial migration of the deciduous second molars just after their eruption (Fig. 1, *A* and *B*) or following the development of dental caries in proximal surfaces of molar teeth. Not a single case substantiated the theory that there is a forward growth of the alveolar frontal

sections during the period of the primary dentition as propounded by almost every textbook author. Similar results are to be seen in Table IV of Goldstein and Stanton[11] on the average length of primary dentitions of different ages.

2. *The Intercanine and Intermolar Width.*—Only minor changes in the transverse dimensions of the upper and lower deciduous arches were apparent during the period of observation. An increment of 0.5 mm. in less than 20 per cent of these cases certainly cannot sustain the general concept of continuously increasing dimensions of the deciduous arches. Identical results with regard to the relatively unchanging width between the ages of 3 and 5½ years can be found in the tables of Lewis,[17] which contain the measurements of 450 different deciduous dentitions of various ages, and in the tables of Goldstein and Stanton[10] for 540 individual cases.




*A.* *B.* *C.*

Fig. 1.—Development of a deciduous dentition *with* spacing. Type I (Case 57).

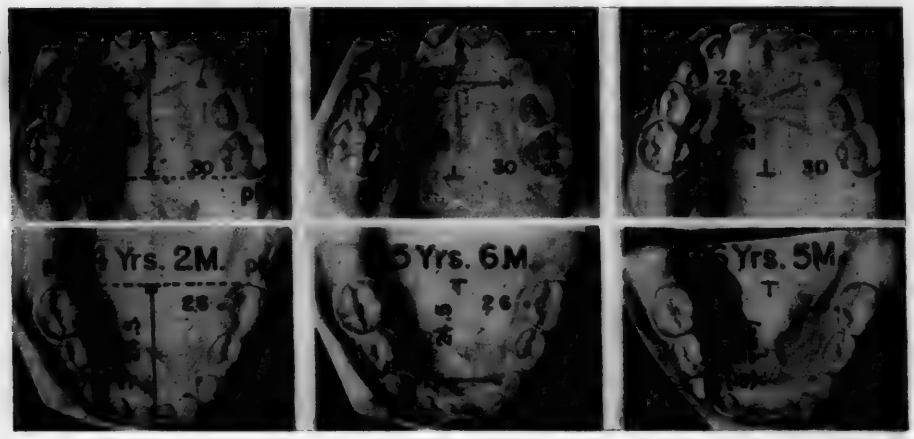
*A.* Model measured at 3 years, 2 months. Note eruption of the second deciduous molars and canines.

*B.* Model measured at 4 years, 5 months. Note increase in intercanine width and decrease in length of arches due to a mesial migration of the second deciduous molars.

*C.* Model measured at 5 years, 4 months. No change in width and length of the arches occurred between *B* and *C*.

3. *Physiological Spacings.*—After complete eruption of the deciduous teeth no physiological spacing occurred. There was no increase in interdental space in those arches with separated teeth and no spacing developed in the arches in which the teeth were in contact. The above findings also show that no adequate extension or expansion of the deciduous arches took place between 3 and 5½ years. This confirms Clinch's[4] observation that he has never seen a case in which the spacing developed after full eruption of all deciduous teeth. The literature does not contain one illustration of the development of a physiological spacing of the deciduous dentition in the same individual between 3 and 6 years. The arrangement of the deciduous teeth is found to exist in either one of two forms; it is spaced (Fig. 1) or closed (Fig. 2), and one does not develop from the other. The spaced form will be referred to as Type I, the closed as Type II.

The distribution of spaces in Type I showed all possible variations; even combinations with Type II in the opposing arch occurred. In 1943 the author reported two frequently observed types of spaces; those between the deciduous upper second incisors and canines and others between the deciduous lower canines and first molars.[2] Models taken from the time of eruption indicated that these diastemata were not the result of functional adaptation but rather due to an inherent pattern. There were already present at the time of eruption of the deciduous cuspids (Fig. 3). These diastemata were interpreted as the so-called "primate spaces" in human deciduous dentition. The occurrence of interlocking long deciduous canines was recognized as further evidence of a primitive feature of the deciduous set. This has been confirmed since by Jones[12] and Seipel.[30*]




*A.* *B.* *C.*

Fig. 2.—Development of a deciduous dentition *without* spaces, Type II (Case 40).

*A.* Model measured at 4 years, 2 months.

*B.* Model measured at 5 years, 6 months. No changes in dimension of the arches are seen.

*C.* Model measured at 6 years, 5 months. Note slight increase in the intercanine width of both lower and upper upon eruption of the mandibular permanent first incisors and slight secondary spacing between upper incisors.

It was not unusual to find cases of Type II which showed slightly crowded anteriors (examples will be demonstrated in Part III, Figs. 2 and 3). As previously stated the deciduous arches without spacing were an average of 1.5 mm. narrower than those with spaces (Table II). Evidently the absence of spaces was not always due only to a greater width of the deciduous anteriors but also to the lack of sufficient alveolar growth, or to a combination of both. Thus most of the cases of Type II may fall under Angle's Class I of malocclusion. A spacing of the upper deciduous anteriors shortly after eruption of the lower permanent ones will be described later.

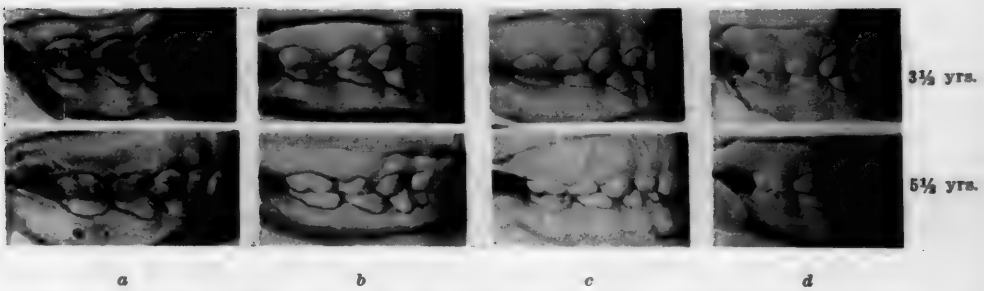
*Seipel,[30] in 1946, in his study of 250 sets of 4-year-old boys quotes the average widths of the interdental spaces. He found the highest average value was 0.85 mm. between the upper II and III and 0.72 mm. between the lower III and IV and termed them "spaces of a primate type."

Etiologically it may be stated that the illustrations of deciduous dentitions of twins and triplets by Korkhaus,[16] Goldberg,[9] and Newton[27] suggest that the presence or absence of spaces in the deciduous dentition may be a hereditary characteristic in that among identical twins both members have either one form or the other.

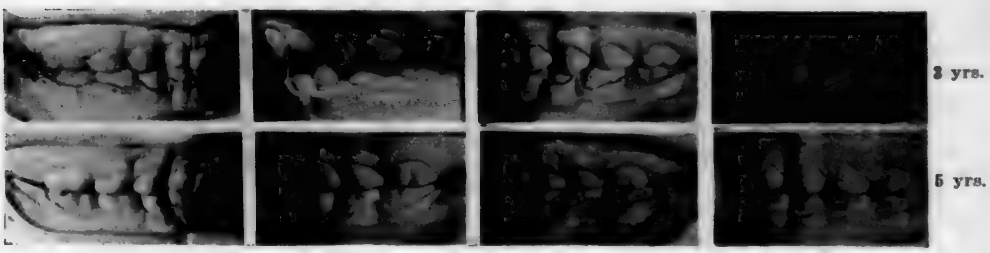
*A.* *B.*

Fig. 3.—*A*. Primate spaces in the maxillary arch between second incisor and canine, and in the mandibular arch between canine and first deciduous molar of a deciduous dentition. Note that the primitive character of the deciduous dentition is accentuated by the fact that the canines project through the plane of occlusion very markedly.

*B* illustrates the manifestation of mandibular primate spaces at the time of eruption of the deciduous canines (Case 50).




*a* *b* *c* *d*




*e* *f* *g* *h*

Fig. 4.—Development of occlusion in the deciduous dentition in 8 cases (Table I) at the ages of 3½ and 5½ years and 3 and 5 years, respectively. Note constancy of the terminal plane and the occlusal relationship of the deciduous canines during the two-year interval.

(*a*)) Case 1
(*b*) Case 27
(*c*) Case 7
(*d*) Case 32
(*e*) Case 31
(*f*) Case 40
(*g*) Case 42
(*h*) Case 57
Type I = *a* - *d*
Type II = *e* - *f*
Distoclusion = *g* - *h*

4. *Anteroposterior Relationship of the Arches.*—It is suggestive from the results of these measurements that no essential changes in occlusion can take place during the period of the completed deciduous dentition. A comparative

study of the models also showed no indication of a physiological mesial shift of the mandible or a forward adjustment of the mandibular teeth after the age of 4. The relationship of the opposing deciduous canines, as well as the position of the distal surfaces of the upper and lower second molars, remained unchanged throughout the period of completed deciduous dentition (Fig. 4). While the terminal plane of the arches was found to be straight in 26 cases (76 per cent) and showed a mesial step in the other 4 cases (14 per cent) this did not develop from one pattern into the other except under environmental influences. The mesial step can be due to a morphological variation in size of the opposing second molars as in instances in which the upper and lower molars are approximately of the same mesiodistal length. Generally the upper molar is shorter mesiodistally than the lower which effects a straight terminal plane.[14, 29, 35] In cases of malocclusion also, there was no change in intermaxillary relationship. The distal terminal step and a cusp-to-cusp occlusion of the deciduous canines in distoclusion remained constant (Fig. 4,*g* and *h*). The distal terminal step is found when the upper deciduous molar occludes mesially to the lower one.

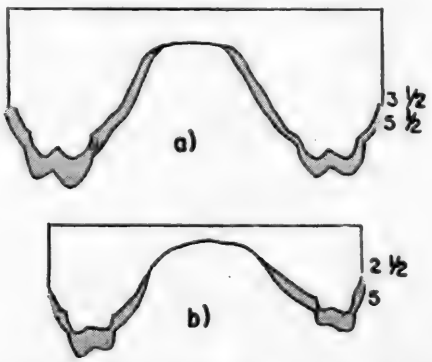



Fig. 5.—Vertical growth of the alveolar processes as shown by transverse diagraphic projections taken at two different ages through the center of the deciduous molars and superimposed on the palate.

*a*. Case 1 at 3½ and 5½ years through center of the second molars.

*b*. Case 50 at 2½ and 5 years through center of first molars.

Although the deciduous dental arches were not involved in any growth changes after their formation, there was, however, vertical growth of the alveolar processes. This increase in height of the alveolar process is demonstrated on diagraphic reproductions (Fig. 5). Sagittal growth of the jaws, as described by Sicher,[31] produced the retromolar areas which develop posteriorly to the arches to make allowance for accessional teeth. Environmental influences such as compression (thumb sucking, etc.), as well as attrition may affect the deciduous dentition. It is possible to misinterpret the attrition of the upper deciduous anteriors as increase in dental height as described by Lewis.[17]

SUMMARY

A study was made to learn the extent and rate of physiological tooth migration and the mechanism which determines the development of occlusion. This is the first of four papers and deals with the development of the deciduous dentition from 3 to 5½ years. Plaster reproductions of the deciduous dental arches of 30 children taken seriatim at various developmental stages were measured and examined for changes in morphology. The findings may be summarized as follows:

1. After the deciduous dental arches were completely formed, their sagittal and transversal dimensions were not altered except when subjected to inadequate environmental influences.

2. Two consistent morphological arch forms of deciduous dentures were found; the arches were either continuously spaced or continuously closed. Spaces in the deciduous denture were not developmental, but congenital. Closed arches were on an average narrower transversally than spaced ones.

3. The spaced arches frequently exhibited two distinct diastemata; one between the mandibular deciduous canine and first deciduous molar and the other between the maxillary second deciduous incisor and deciduous canine. These were interpreted as "primate spaces."

4. The terminal plane of the arches in occlusion remained constant.

5. Vertical growth of the alveolar processes concomitant with the development of the successional tooth germs and sagittal growth concomitant with the development of the accessional tooth germs was observed.

6. The present concept of physiological changes of the deciduous dental arches through spacing and through mesial shifting of the mandibular teeth was not confirmed by the above observations.

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# VERTICAL FORCES USED DURING CHEWING OF FOOD

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NUMEROUS investigators have been interested in the actual forces used during mastication, but lack of suitable equipment has prevented direct measurement of these forces. Indirect measurements have been made,[1-6] but recently technics have been introduced which are adaptable to the direct measurement of the masticatory forces and the present paper described such measurements.

The method employed is an extension of the scheme used by Howell and Manly.[7] The principle involves a variable inductance type of strain gauge which has been made sufficiently small so it can be inserted under an artificial tooth to form a bite element. Fig. 1 shows individual bite elements of this type and a set of dentures in which four of these bite elements have been incorporated on the left side of the upper denture. The bite elements are connected to associated electronic equipment and a strip-chart recorder by means of very thin wires so that measurements can be taken without interfering with normal chewing or with the comfort of the test subject. The forces are recorded as deflections on a strip chart. An example of the kind of record that is obtained is shown in Fig. 2 where the complete record of the chewing of a mouthful of raisins is shown. Simultaneous measurements on more than one tooth are possible when multichannel electronic circuits and a multichannel recorder are used. Fig. 3 shows how simultaneous measurements can be made on three different teeth.

The details of the electronic equipment used in this work are substantially different from those previously described, but the general method is similar. Calibrating technics have been worked out so that all of the data which have been taken are quantitative.

The masticatory forces were measured in a 48-year-old man who had been wearing complete dentures for a period of five years. No instructions were given to the subject during the experiment except that he was asked to chew

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on the test side only. Three foods having a wide range in physical properties were tested. Peanuts represented the brittle type of food, while shredded coconut and raisins served as examples of the tougher foods. Measurements were taken in duplicate from the initial bite to swallowing.

Fig. 1.—Individual bite elements and a set of dentures with four bite elements inserted on the left side of the upper denture.

## RESULTS AND DISCUSSION

The data were evaluated as shown in Table I. There appeared to be little difference in the average force exerted on a tooth when the different types of food were chewed. The sum of the mean forces on the two premolars and the first molar amounted to 3.7 kg., 4.1 kg., and 4.9 kg. for peanuts, coconut, and raisins, respectively. The maximal force recorded, 7.2 kg., was found on the first molar during the chewing of raisins. The forces were generally of a smaller magnitude than expected.

TABLE I

FORCES USED DURING CHEWING OF A MOUTHFUL
(Measured in Kilograms)

| FOOD AND TOOTH TESTED | TEST NO. | NO. OF CHEWS | | MEAN | | MEDIAN | | MAXIMUM | |
|---|---|---|---|---|---|---|---|---|---|
| | | I | II | I | II | I | II | I | II |
| Peanuts: | | | | | | | | | |
| First premolar | | 70 | 70 | 0.4 | 0.4 | 0.3 | 0.3 | 1.3 | 1.3 |
| Second premolar | | 61 | 64 | 1.2 | 1.0 | 0.9 | 0.7 | 3.0 | 2.5 |
| First molar | | 38 | 40 | 2.5 | 1.9 | 2.4 | 1.7 | 4.7 | 4.0 |
| Coconut: | | | | | | | | | |
| First premolar | | 52 | 51 | 0.7 | 0.7 | 0.6 | 0.7 | 1.5 | 1.8 |
| Second premolar | | 40 | 44 | 1.1 | 1.3 | 0.7 | 1.1 | 2.7 | 3.5 |
| First molar | | 48 | 33 | 2.1 | 2.3 | 1.9 | 2.3 | 5.2 | 4.5 |
| Raisins: | | | | | | | | | |
| First premolar | | 53 | 56 | 1.0 | 0.9 | 0.8 | 0.7 | 2.8 | 3.0 |
| Second premolar | | 67 | 59 | 1.3 | 1.5 | 0.9 | 1.3 | 4.0 | 3.8 |
| First molar | | 53 | 50 | 2.8 | 2.3 | 2.6 | 2.0 | 7.2 | 6.5 |

TABLE II

PERCENTAGE DISTRIBUTION OF FORCES ON DIFFERENT TEETH
(Average of Two Tests)

| FOOD | FIRST PREMOLAR | SECOND PREMOLAR | FIRST MOLAR |
|---|---|---|---|
| Peanuts | 10.8 | 29.8 | 59.4 |
| Coconut | 17.1 | 29.2 | 53.7 |
| Raisins | 20.4 | 28.6 | 51.0 |

Table II shows the percentage distribution of the masticatory forces on the different teeth. It is interesting that when the tougher foods, coconut and raisins, were chewed, the first premolar was used to a greater extent than when peanuts were chewed. The forces on the second premolar remained remarkably constant for all the foods. Apparently, this tooth was used consistently at full capacity.

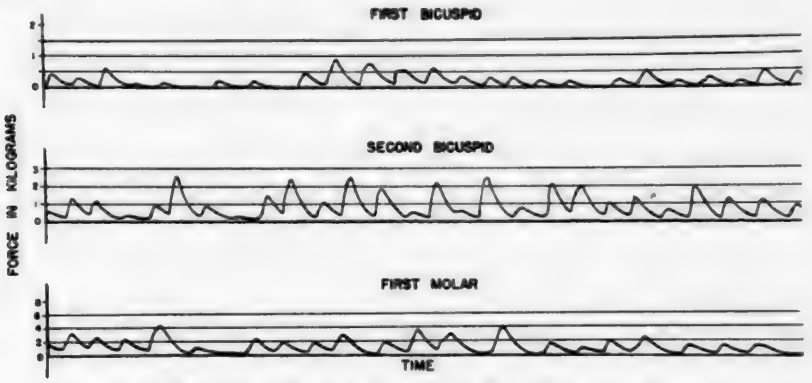


Fig. 2.—Recordings obtained on different teeth in chewing peanuts.

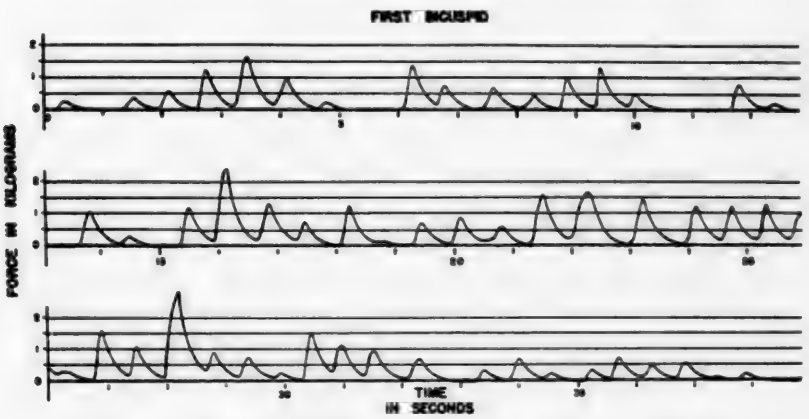


Fig. 3.—Complete recording of the chewing of a mouthful of raisins.

A discussion of masticatory forces would be incomplete without a consideration of the occlusal area on which the force is exerted. A force concentrated in a small area will cut or crush more effectively than the same force applied over a large area. Therefore, it would be important if the force per

unit area, or the pressure, could be calculated. The area to consider would not be the occlusal area of the teeth because some of this area is not used during mastication, but, rather, it would be that part of the occlusal area which is in contact, or nearly in contact, with the opposing teeth during mastication.[8] This area would vary depending on whether the teeth occlude in centric or in lateral excursion. The movements of the jaw were not studied in this experiment, but the occlusal contact area of the bite elements in centric occlusion was measured, using the method of Yurkstas and Manly.[8] The figures are given in Table III.

TABLE III

AREA OF OCCLUSAL CONTACT IN SQUARE MILLIMETERS

| TOOTH TESTED | AREA OF CONTACT | PERCENTAGE OF TOTAL AREA |
|---|---|---|
| First premolar | 3.5 | 14.6 |
| Second premolar | 6.5 | 27.1 |
| First molar | 14.0 | 58.3 |

The distribution of pressure was calculated and the results are given in Table IV. The calculation is based on the assumption that the teeth contact each other in centric occlusion during mastication, and may therefore not be entirely accurate. It will be seen that the pressure is increased on all the teeth when peanuts and raisins are compared, and that the increase is smallest for the first molar and greatest for the first premolar. The first premolars appears to be the most efficient cutter when tough food is chewed.

TABLE IV

FORCE (F) IN KILOGRAMS AND PRESSURE (P) IN KILOGRAMS PER MM.$^2$ ON INDIVIDUAL TEETH

| FOOD | FIRST PREMOLAR | | SECOND PREMOLAR | | FIRST MOLAR | |
|---|---|---|---|---|---|---|
| | F | P | F | P | F | P |
| Peanuts | 0.4 | 0.114 | 1.1 | 0.169 | 2.2 | 0.157 |
| Coconut | 0.7 | 0.196 | 1.2 | 0.185 | 2.2 | 0.157 |
| Raisins | 1.0 | 0.286 | 1.4 | 0.208 | 2.5 | 0.179 |

The results presented in this study are preliminary, and only limited conclusions can be drawn. It seems that the range of force which a denture wearer uses during mastication is small. Mastication of tough food is handled either by increasing the masticatory force, or by shifting the food forward to the smaller premolars. The forward shifting would result in increased pressure even if there was no increase in force.

The authors wish to express their appreciation for the helpful suggestions given by Dr. R. S. Manly in initiating the work, and for the generous assistance given by Captain John D. Connor, USN, in carrying out the tests.

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# PRELIMINARY REPORT IN THE USE OF XYLOCAINE AS A LOCAL ANESTHETIC IN DENTISTRY

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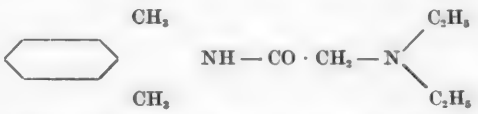
THERE is no purpose in introducing a new drug to the science of the healing arts unless it possesses superior qualities over drugs hitherto employed for similar usage. In Xylocaine we have obtained a drug for local anesthesia that has given evidence that it has definite advantages over procaine or other allied drugs.

Xylocaine was discovered in 1943 by the Swedish chemists Löfgren and Lundquist.[1] The drug was a result of a series of experiments with basic anilids which were widely different in structure from the cocaine-procaine group. Further elaborations and developments were pursued by the Astra Pharmaceutical Company of Sweden. The toxological and pharmacological investigation was conducted by Goldberg.[2]

Xylocaine chemically is composed as:

-ω- diethylamino - 2.6 - dimethylacetanilid

or structurally as:




Xylocaine seems to be more selective for the sensory and parasympathetic nerve fibers and less specific for the motor nerves. Herein is a pharmacological advantage over procaine. Xylocaine in itself is a more potent anesthetic so that epinephrine should be added with discretion.[3] The toxicity for local tissue reaction and systemic manifestations is particularly low in comparison to other local anesthetics. The risk of toxic reactions is incurred in all of our local anesthetics. These reactions are manifest by symptoms of irritations from the central nervous system and may be accompanied by respiratory and circulatory collapse. The symptoms may be mild, with a tendency toward dizziness or syncope or ranging to those of a more serious nature with unconsciousness and convulsions. In the course of study of Xylocaine, Gordh[4] has noticed only two cases of toxic reactions after deliberate overdosing (1.35 and 3.0 Gm., respectively, as compared with 4 mg. used in dentistry). The treatment of these states consists of the ordinary collapse therapy measures, with maintenance of free air passages, oxygen, artificial respiration and lowered head end, and, in case of convulsions, intravenous anesthesia using a barbituric acid derivative of short duration. These two cases are mentioned only as extremes after thousands of injections by various investigators. It also illustrates that the usual treatment of such toxic states is effective in the event of Xylocaine poisoning. Among other reactions observed in the course of Gordh's investigation was a transient

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nausea after the anesthesia that occurred in two cases. Otherwise no noteworthy effects on the pulse, blood pressure, or respiration has been observed. It has been observed that there have been definitely less blood pressure falls with Xylocaine with or without epinephrine than with other drugs.[5] In cases where considerable amounts are injected, the combination with epinephrine should always be used. It may be pointed out that Braun (1925) discouraged the use of the ordinary clinical dosage of procaine (0.5 to 1.0 Gm.) without the addition of epinephrine on the ground of the risk of toxic reaction. The rate of absorption factor of any local anesthetic must be considered. Gordh[4] further reported that in cases where toxic reaction had previously occurred in connection with procaine and allied drugs, Xylocaine can be used without entailing similar symptoms. As Xylocaine can be used without epinephrine, the local anesthetic has been employed in cases of sensitivity to that drug.

## INDICATIONS AND DOSAGES

Xylocaine is available in 0.5 per cent, 1 per cent, and 2 per cent solutions. At present the epinephrine ratio ranges are 1:50,000 and 1:100,000. In dentistry the recommended dosage is a 2 per cent solution of 1:100,000 epinephrine ratio. In instances where anesthesia is difficult, a 2 per cent solution with 1:50,000 epinephrine is used. However, in most instances the 2 per cent 1:100,000 solution is sufficient. Carpules containing 1.8 c.c. and 2.4 c.c. of solution are supplied for dentistry.

Xylocaine without epinephrine is indicated: (1) when there is oversensitivity to epinephrine, especially in thyrotoxicoses and certain other conditions in which epinephrine might have a bad effect, such as angiospasm, arteriosclerosis, and diabetes, when relatively large amounts must be injected; (2) in anesthesia of the fingers and toes; and (3) when the surgical treatment called for is very brief.

At present Xylocaine is being used experimentally in general surgery, urology, otolaryngology, obstetrics, and dentistry. The commonly employed methods of administering Xylocaine are, as with procaine, infiltration, conduction, and terminal anesthesia. At the Johns Hopkins Hospital, the Department of Obstetrics has used Xylocaine in approximately 250 conduction blocks to date. The average dosage in saddle-block spinal anesthesia used is 30 mg. (1½ c.c.).

## ADVANTAGES OF XYLOCAINE AS COMPARED TO PROCAINE

| *Xylocaine* | *Procaine* |
|---|---|
| 1. Rapidity of analgesia and anesthesia. | 1. "It is not particularly active. . . . it is to be classed among the weaker local anesthetics. . . . but it has a strikingly low toxicity. This property, together with its mild effect on the tissues, has chiefly contributed to the success of procaine."[6] |
| 2. Stability. It is not decomposed either in strongly acidic or strongly alkaline solutions, not even if boiled. The trade product of Xylocaine has an osmotic pressure so adjusted that the solution does not cause hemolysis or plasmolysis. | 2. Procaine as an ester is not stable in an aqueous solution. Most local |

3. When allergies and contraindications to epinephrine exist, straight Xylocaine may be used with greater abandon than can procaine.
4. Extremely low incidence of side reactions.
5. Anesthesia profundity.
6. More selectivity to sensory and parasympathetic nerve fibers and less selectivity to the motor nerves. Hence lessened discomfort to the patient subjectively.
7. Lessened likelihood of "hangover," itching, and paresthesia when the drug is wearing off.
8. In infiltration it is more expansive to adjacent teeth. In mandibular blocks, it is more effective in the premolar area.
9. It has possibilities of being a surface or topical anesthetic.
10. In cases of toxic reactions to procaine, Xylocaine is well tolerated.
11. In instances of procaine dermatitis, Xylocaine is indicated.

anesthetics hitherto used can scarcely stand boiling in an aqueous solution.

3. Procaine should not be used without a considerable addition of epinephrine as applied to dental procedures.
4. When combined with epinephrine, procaine not infrequently gives rise to unpleasant side reactions on the general condition of the patient. (The fault cannot always be laid at the door of epinephrine alone.)[6]

### DISADVANTAGES

The duration of anesthesia seems to be longer than procaine. To the ambulatory patient in dentistry this might be an inconvenience.

### OBSERVATIONS

To date the author has utilized Xylocaine in all types of general practice of dentistry in approximately 1,000 cases. The striking fact noted is the smoothness of anesthesia, rapidity, profundity, and the subjective reactions of the patients. Side reactions and postoperative reactions are rare. Many patients were under psychiatric treatment at the Henry P. Phipps Institute at the Johns Hopkins Hospital. In some instances where procaine anesthesia had failed, cases including porcelain jacket preparations and cervical procedures in operative dentistry, Xylocaine accomplished profound anesthesia. There were 14 instances in which the same operative procedure on the same tooth was attempted without success before Xylocaine was employed. Table I gives the results of 994 injections.

It is to be noted that as in procaine the conduction and terminal injections are more effective than with the infiltration injections. When the author first started using Xylocaine, the 2 per cent carpules with epinephrine 1:100,000 were not available. Experiments mixing epinephrine with Xylocaine were con-

TABLE I

| METHOD OF INJECTION | TIME OF INJECTION | SYMPTOMS OF ANALGESIA (SECONDS) | ANALGESIA TIME (MINUTES) | ANASTHESIA TIME (MINUTES) | DURATION (HOURS) | NUMBER OF INJECTIONS | SUCCESS OF INJECTIONS | FAILURES | PER CENT OF SUCCESS | SIDE REACTIONS |
|---|---|---|---|---|---|---|---|---|---|---|
| Infiltration | xx | 10 | 1 | 2 | 2 | 390 | 379 | 11 | 97 | nil |
| Tuberosity and zygomatic | xx | 30 | 1½ | 3 | 3 | 248 | 243 | 5 | 98 | nil |
| Mandibular | xx | 30 | 2 | 3 | 3-4 | 356 | 346 | 10 | 97 | nil |
| Total | | | | | | 994 | 968 | 26 | 97.3 | |

ducted. Solutions of 0.5 per cent, 1 per cent, and 2 per cent with epinephrine ratios ranging from 1:10,000, 1:20,000, and 1:40,000 were used. Amounts originally given varied from 1 c.c. to 2 c.c. It was during this early experimentation that the anesthesia failures occurred. Since employing the ready prepared carpules of 2 per cent 1:100,000, the per cent of failures has been nil.

In only two cases was anesthesia a complete failure. The first patient was given 1.8 c.c. of 2 per cent 1:100,000 solution and, as pain was still present, 2 c.c. of 2 per cent 1:50,000 solution was given. In two previous attempts with procaine, anesthesia was not obtained. The tooth operated upon was an upper canine with an eroded area on the cemental surface at the distal aspect. This tooth acted as an abutment for a removable partial prosthesis with a free-end saddle on the same side. The second case was for the extraction of a lower left third molar. The tooth was extremely tender to percussion prior to extraction and the patient had had adenitis for 24 hours previous to the extraction. The tooth was very mobile and the patient presented a periodontosis throughout the dental arches. Anesthesia failed with a 2 per cent 1:100,000 solution and again with a 2 per cent 1:50,000 solution.

Xylocaine without epinephrine, in the author's experience, is only practical for mandibular injections. In these cases, success was encountered with 1 c.c. of a 1 per cent solution. Surface anesthesia of peritoneum and mucous membranes require further investigation.

TABLE II

| EPINEPHRINE CONC. | AMOUNT IN ML. (C.C.) | NUMBER OF CASES | GENERAL REACTION |
|---|---|---|---|
| 1:100,000 | 0.8 to 1.2 | 83 | 0 |
| 1:100,000 | 1.5 | 165 | 0 |
| Total | | 248 | 0 |

The opportunty for trial in pulp extirpation was presented three times during the testing of the drug. In these cases a 2 per cent 1:50,000 solution was employed and with excellent results. In the first case presented, a 2 per cent 1:100,000 solution of epinephrine was first used. As pain still persisted, a 2 per cent 1:50,000 solution was given and anesthesia was complete. Clinically, these three cases presented no putrescence, suppuration, or gangrene of the pulp

but venous hyperemia with a good blood supply in which the prognosis was negative. However, Bremer, Ekmanner, Persson, and Strandberg[6] have some interesting data for pulp removal. (Table II.)

In experiments with Xylocaine by Lloyd and Blythe of the U. S. Marine Hospital in Baltimore, approximately 150 cases were tested by using procaine on one side and Xylocaine on the other side simultaneously.[7] Both infiltration and conduction technics were employed. Their findings show that Xylocaine produced anesthesia with greater rapidity and that Xylocaine took approximately one hour longer to wear off. In all cases their patients were not informed that an experiment was being conducted. Furthermore, the patients' subjective symptoms were more pronounced with Xylocaine than with procaine. The author's personal observation from clinical usage would substantiate the findings of Lloyd and Blythe.

## AVAILABILITY

To date Xylocaine is not available to the fields of medicine and dentistry at large in this country. It is being used experimentally by teaching institutions here at present. The present reports are that ninety per cent of the Swedish dentists are using Xylocaine in preference to procaine today. As stated by Bremer, Ekmanner, Persson, and Strandberg,[6] the above properties of Xylocaine: rapid and effective anesthesia results . . . alone make it preferable to procaine. The most important advantages from the standpoint of being practical are to be found in the circumstances that it gives rise to very few side reactions and "hang-over," and that it is easily tolerated even by sensitive patients. The introduction of Xylocaine must therefore be designated as a great advance. Our experience with the agent, after careful testing, has convinced us that procaine as a dental injection anesthesia will be replaced by Xylocaine.

## SUMMARY

1. The anesthetizing effect of Xylocaine clearly excels that of procaine. This is particularly evident in stubborn cases.

2. The rapidity with which it takes effect is considerably greater than that of procaine.

3. In infiltration it is more expansive to adjacent teeth. In mandibular blocks, it is more effective in the premolar area.

4. Local tissue reaction and general side reactions are extremely rare.

5. In contradistinction to procaine, Xylocaine gives anesthesia effect without the addition of epinephrine.

6. Postinjection reactions are rare.

7. The selectivity of the drug to the sensory and parasympathetic nerve fibers in preference to the motor nerves make it more comfortable to the patient subjectively.

8. In cases of procaine dermatitis, Xylocaine can be used without reaction.

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DISCUSSION

Robert A. Hingson, M.D., Department of Obstetrics, Johns Hopkins University and Hospital, Baltimore, Md.—In the clinical assay of the safety and efficiency of a new anesthetic agent, it is valuable to compare results from the fields of dentistry and medicine. In such a cooperative analysis the two professions can rekindle the historical principles upon which dentistry and medicine together developed the first uses of anesthesia in the fifth decade of the nineteenth century.

Simultaneously the Departments of obstetrics, urology, and dentistry of the Johns Hopkins Hospital instituted the use of Xylocaine as a local anesthetic. Dr. Gruber's report records its satisfactory use herewith in more than 990 cases in dentistry. Our report, which will be published in the medical literature, includes its application in 250 cases. For anesthesia in these cases we have used 1 per cent Xylocaine with and without epinephrine, 2 per cent Xylocaine with and without glucose, and 2 per cent Xylocaine with and without epinephrine, for caudal and continuous caudal, lumbar peridural and continuous peridural analgesia, spinal and continuous spinal anesthesia, saddle-block hyperbaric spinal anesthesia, and local infiltration anesthesia. The maximum total dosage administered to any one patient was 1,100 mg. in continuous lumbar peridural anesthesia for nephrectomy and dissections of adhesions under diaphragm in a three-hour operation on a 66-year-old patient. We have been impressed by the low incidence of side reaction such as nausea, vomiting, and hypotension in cases where Xylocaine was used. We substantiate the report of Drs. Gruber, Gordh, and others, which claims a rapid and intense action of Xylocaine. Thus far in our series there has been no major complication.

# COMPARISON OF GOLD ALLOY AND STAINLESS STEEL WIRE FOR ORTHODONTIC ARCHES AND SPRINGS

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MOST gold alloy wires for arches and springs are of the type which can be softened by quenching from a heated condition. A subsequent slow cooling from a temperature of about 850° F. will increase the strength and proportional limit.

Stainless steels which are used for arches and springs are of type 300 series generally of about 18 per cent chromium with 8 per cent nickel. To obtain the necessary strength the wires are cold drawn since this composition does not harden by heat treatment.

Since both gold alloy and stainless steel wires are satisfactory for use in the mouth from the standpoint of tissue tolerance, tarnish, and corrosion resistance, it is desirable to consider the effects of other physical properties on their usefulness. The measured physical properties which appear to be the most important are modulus of elasticity, elastic limit, and modulus of desilience.

*Tensile vs. Bending Characteristics*

For a wire used as a spring in transverse bending, tensile and bending characteristics should be determined by bending tests since tensile tests and bending tests do not in many cases show concordant properties on the same material. If the tensile properties are analyzed in determining the usefulness of materials, wrong conclusions will frequently be reached if the materials are to be used in bending.

An example giving tensile and bending measured properties will illustrate this discrepancy. A wire which was popular with orthodontists prior to the adoption of A.D.A. Specification No. 7 does not meet the specifications. This will be called wire "A." A new wire was developed which did meet the specifications. This will be designated as wire "B." Wire B was submitted to orthodontists who found it inferior to Wire A in their clinical use. This was surprising in view of its better tensile properties and prompted an investigation by the author as to the cause. Tensile and bending tests were made. The results are shown in Table I.

While the data given in Table I does not give conclusive evidence why orthodontists preferred Wire A, it does show how wrong conclusions would be formed if properties are judged on tensile data. The tensile figures for Wire B are as a whole definitely superior to those of Wire A whereas in transverse bending Wire A has a slight advantage in the oven cooled condition due to its lower modulus of elasticity. It will be noted that the bending elastic limit for

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Wire A is higher than the U.T.S. The data has been checked by two different types of bending tests with concordant results. Also the moduli of elasticity differ in tensile and bending tests, higher for tension in Wire B and lower for Wire A. If there is a satisfying theory to explain these facts, I do not know of it.

TABLE I

PHYSICAL PROPERTIES: TENSILE AND BENDING

| | | | WIRE A | | WIRE B | |
|---|---|---|---|---|---|---|
| | | | QUENCHED | OVEN COOLED | QUENCHED | OVEN COOLED |
| Tensile: | | | | | | |
| U. T. S. | | lbs./sq. in. | 104000 | 143000 | 125000 | 186000 |
| Proportional limit | | lbs./sq. in. | 76000 | 107000 | 101000 | 151000 |
| Elongation | | per cent | 15.0 | 1.7 | 12.0 | 4.7 |
| Modulus of elasticity | | lbs./sq. in. x $10^6$ | 13.0 | 14.5 | 17.0 | 17.3 |
| Modulus of resilience | | lbs./in. cubed | 222 | 395 | 300 | 659 |
| Transverse Bending: | | | | | | |
| Elastic limit | S | lbs./sq. in. | 117000 | 181000 | 131000 | 181000 |
| Modulus of elasticity | E | lbs./sq. in. x $10^6$ | 13.2 | 14.6 | 15.3 | 15.9 |
| Modulus of resilience | R | lbs./in. cubed | 519 | 1122 | 561 | 1030 |

S was determined on 2 inch long specimen with bending moments applied at both ends.
E was determined from a cantilever loaded at the end.
R was calculated by formula $\frac{S^2}{2E}$.

*Bending Characteristics of Gold Alloy vs. Stainless Steel*

One may look upon the modulus of elasticity as a measure of stiffness and elastic limit as the maximum usable stress, while modulus of resilience combines elastic modulus and elastic limit to give a measure of usable work which can be expected from materials having differing physical properties. All measures of elastic modulus and elastic limit used in this paper were determined by bending tests unless otherwise noted. The modulus of resilience is represented graphically on the stress strain plot by the area under the curve up to the elastic limit.

Typical data and curves of stress deflection for gold alloy and stainless steel cantilevers having the same elastic limit are shown in Table II.

TABLE II

| | | | | GOLD ALLOY | STAINLESS STEEL |
|---|---|---|---|---|---|
| Modulus of elasticity | | E | lbs./sq. in. x $10^6$ | 14.6 | 25.0 |
| Elastic limit | | S | lbs./sq. in. | 180,000 | 180,000 |
| Length of cantilever | = | L | inches | 1 | 1 |
| Diameter of wire | = | d | inches | .020 | .020 |
| Deflection $= \frac{2SL^2}{3Ed}$ | = | e | inches | .411 | .240 |
| Modulus of resilience $R = \frac{S^2}{2E}$ | | | lbs./in. cubed | 1110 | 648 |
| Efficiency as a spring | | | per cent | 100 | 58 |

Therefore a gold alloy under conditions stated can do 70 per cent more work than stainless steel. This is calculated from the moduli of resilience as follows:

$$\frac{1110-648}{648} \times 100 = 70+ \text{ per cent.}$$

This can be stated in another way as follows: If a spring of gold wire is found satisfactory to accomplish an objective, i.e., has a correct pressure and deflection, a steel wire of the same size will, for equal pressure, have a smaller deflection by the ratio of $\frac{648}{1110}$ or 58 per cent. The adjustment of such stainless steel spring would need be at more frequent intervals to accomplish the same movement of a tooth.

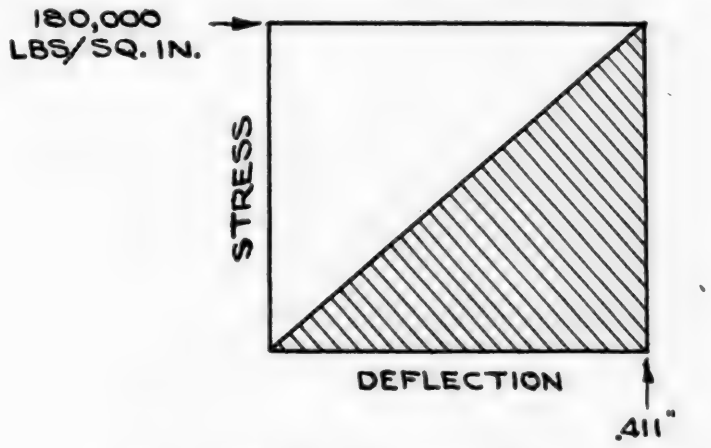


Fig. 1.—Gold alloy stress deflection curve.

*Wire Diameter for Equal Deflection*

The same deflection for equal force can be obtained in both gold alloy and stainless steel wire if the steel wire is smaller in cross section. Since deflection in a cantilever varies inversely as the modulus of elasticity and the fourth power of the diameter, the formula for calculating of wire size is:

$$d_s = \left(\frac{E_g}{E_s}\right)^{1/4} d_g \quad \text{(Equation 1)}$$

where subindices mean "g" for gold alloy and "s" for stainless steel.

Typical values are as follows:

$E_g = 14.6 \times 10^6$ lbs. per sq. in.
$E_s = 25.0 \times 10^6$ lbs. per sq. in.

$$\left(\frac{E_g}{E_s}\right)^{1/4} = .874 \quad \text{(Equation 2)}$$

$$d_s = .87\ d_g$$

If gold wire of .020 inch diameter gave desired spring characteristics, a steel wire must be smaller in diameter to give the same spring characteristics. The diameter of steel wire would be $.020 \times .87 = .0174$ inch.

*Wire Diameter—Stress*

The smaller diameter in the steel wire affects its stress for same deflection and force and requires that it have a higher elastic limit as compared with a gold alloy wire. Since the stress is inversely proportional to the cube of the diameter we may write the formula:

$$S_s = S_g\left(\frac{d_g}{d_s}\right)^3$$

Substituting the value of $d_s$ from Equation 2 we have:

$$S_s = S_g\left(\frac{d_g}{.87d_g}\right)^3$$

$$S_s = 1.5\ S_g$$

Therefore the stress in a steel wire to have bending characteristics the same as for a gold alloy wire, the elastic limit must be 1.5 times that for a gold wire.

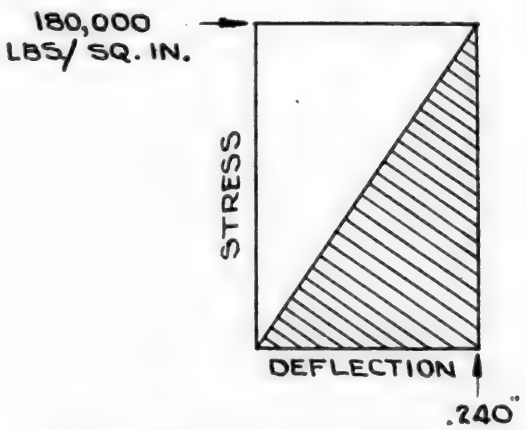


Fig. 2.—Stainless steel stress deflection curve.

The gold alloy Wire A (composition gold 64 per cent, platinum 10 per cent, silver 15 per cent, copper 11 per cent) has in transverse bending an elastic limit of 181,000 lbs. per sq. in. in the oven-cooled condition. A steel wire to be an equivalent spring would need to have an elastic limit of 181,000 × 1.5 = 272,000 lbs. per sq. in. The highest elastic limit which we have found in stainless steel wire was 210,000 lbs. per sq. in.

*Soldering*

When stainless steel wire is to be soldered the heat of soldering reduces the elastic limit. The amount of reduction will depend upon the time and the temperature of the soldering operation.

Stainless steel having an ultimate tensile strength of 311,000 lbs./sq. in. (the strongest we have measured) was reduced to 119,000 lbs./sq. in.-UTS by soldering it with 650 fine gold solder. Other sections of this same wire were heat treated to obtain the same U.T.S. The elastic limit in bending was

measured and after the tempering treatment found to be 109,000 lbs. per sq. in. which is far below the 181,000 lbs./sq. in. obtainable in heat-treated gold alloy wire. The results are calculated in Table III.

TABLE III

BENDING CHARACTERISTICS

| | | | GOLD ALLOY HEAT TREATED OVEN COOLED | STAINLESS STEEL AS SOLD | STAINLESS STEEL AFTER SOLDERING |
|---|---|---|---|---|---|
| Elastic limit | S | lbs./sq. in. | 181,000 | 183,000 | 109,000 |
| Modulus of elasticity | E | lbs./sq. in. x $10^6$ | 14.6 | 25.0 | 25.0 |
| Modulus of resilience | R | lbs./in. cubed | 1122 | 670 | 238 |
| Efficiency as a spring | | per cent | 100 | 60 | 21 |

## CONCLUSION

Due to the greater modulus of resilience of gold alloy wire designed for spring purposes, the conclusion is easily reached that gold alloy wires will serve orthodontists for arches and springs better than the best stainless steel wires. The advantage of gold alloys which can be heat treated after soldering operation, is especially pronounced as compared with stainless steel.

# STUDIES ON THE INCIDENCE AND CAUSE OF DENTAL DEFECTS IN CHILDREN

## VIII. The Deciduous Molars: Caries Incidence

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STUDIES on the incidence of caries of the deciduous molars have been reported by a limited number of investigators. The few accounts from different parts of the world emphasize the low resistance of these teeth to dental decay. Some years ago Toverud[1] declared that according to information received from the school dental clinics of Oslo, Norway, 94 per cent of the deciduous molars were carious. Techow[2] in his observations on a group of 6-year-old children, 592 boys and 620 girls, noted that of the deciduous dentition the molars were the teeth most liable to decay. He found the upper molars the most susceptible to caries. Investigations by McCall[3] on New York City school children ranging in age from 2 to 6 years inclusive brought out similar deductions. As a result of studies carried out on a group of New York City girls of various races and social strata, from preschool to high school age, Dwyer[4] came to the conclusion that the lower molars (both first and second) were more frequently affected by caries than were the upper. This fact was also noted by Cohen[5] in his examination of 2,554 Minneapolis public school children from 2 to 15 years of age. He also concluded that, in general, children of higher social levels have better deciduous teeth. Observations as to the susceptibility to caries of the deciduous molars carried out at Washington University[6] demonstrated that all eight deciduous molars were carious in 25 per cent of the patients at the age of 6. The number of children examined was not given.

### THE PRESENT INVESTIGATION

### SOURCE OF MATERIAL AND METHOD

This report deals with findings accumulated in a study of a mixed group of 2,211 children of Newark, New Jersey. One thousand, one hundred and sixteen of those investigated were boys (681 white, 435 Negro). The balance of 1,095 were girls (615 white, 480 Negro). Their ages ranged from 4 to 14 years inclusive. Various nationalities were included in the group and the socioeconomic status of those examined was slightly above the average (in the white children especially). One group of 1,005 white pupils lived in a suburban section of the city with many trees, open spaces for basking in the sun, and with good modern homes. The remaining, 1206 white and Negro children inhabited a downtown district with narrow streets, heavy traffic, few trees and lawns, and with homes practically joined together (more like tenement lodgings), and with little or no garden space. Theirs could not be called up-to-date dwellings.

The majority of those having deciduous molars belonged to the 4- to 10-year age groups. As would be expected, the older children showed a smaller percentage of such teeth, as their loss through caries or normal exfoliation was

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accomplished. To be sure, a fairly large number of deciduous molars persisted in many mouths up to adolescence. In many of these cases these teeth were found to be free from fillings and caries.

A total of 3,891 second deciduous molars and 3,485 first deciduous molars was studied. The mouth mirror, explorers, college pliers, cotton pellets, and scalers were used in the examination. The x-ray was not employed.

Because of claims that no one can prove that cavities involving more than one surface of the molars (both permanent and deciduous) have not originated on the occlusal aspects of these teeth, it was considered best in this study to group all decayed molars together at first, regardless of the surface on which the cavity occurred. Filled deciduous molars were not rated in establishing the extent of caries prevalence. As there was no definite way of ascertaining which were filled because of caries and which had undergone operative procedures in order to "prevent" their decay, it was concluded that they should be tabulated separately. Deciduous molars were classified as carious if at the time of examination they showed evidence of decay by the presence of an actual cavity containing decomposed tooth substance. Those with fissures but without true cavity formation were not classed as carious, but were grouped apart. As the figures were determinated on the basis of findings revealed by deciduous molars present, missing molars were not taken into consideration.

TABLE I

AGE DISTRIBUTION IN THE WHITE AND NEGRO CHILDREN WITH DECIDUOUS MOLARS PRESENT AT TIME OF EXAMINATION

| AGE AT LAST BIRTHDAY | WHITE BOYS | WHITE GIRLS | NEGRO BOYS | NEGRO GIRLS |
|---|---|---|---|---|
| 4 | 8 | 5 | 6 | 2 |
| 5 | 41 | 36 | 24 | 40 |
| 6 | 59 | 54 | 29 | 37 |
| 7 | 43 | 47 | 46 | 51 |
| 8 | 79 | 61 | 54 | 48 |
| 9 | 56 | 53 | 33 | 37 |
| 10 | 67 | 47 | 39 | 26 |
| 11 | 40 | 26 | 17 | 24 |
| 12 | 20 | 22 | 8 | 13 |
| 13 | 9 | 3 | 6 | 5 |
| 14 | 4 | 4 | 2 | 0 |
| Total | 426 | 358 | 264 | 283 |

## THE FIRST DECIDUOUS MOLARS

### ANALYSIS OF FINDINGS

Of the total number of 1,869 first deciduous molars studied in the white boys and girls, 782 (41.8 per cent) were found to be carious (Table II) (filled teeth and those with fissures were not included). The Negro boys and girls (Table II) revealed a much lower incidence: a little over 29 per cent of the 1,616 first deciduous molars considered were found involved (here again filled teeth and those with fissures were not included). A question which keeps recurring is what factor is responsible for a greater resistance to caries of these teeth in the Negro children. Dietary influences, habits of oral hygiene, social and economic status were potentialities fully considered but without a suggestive answer.

The white boys showed a smaller percentage of carious first deciduous molars than the white girls (Table II). In the case of the Negro children the reverse was demonstrated, namely, the caries prevalence was lower in the girls (Table II). The relation of sex to caries occurrence appeared a tangled problem. No positive conclusions could be made. The capacity for resistance to the attacks of dental decay was differently manifested in the males and females of the two races.

TABLE II

INCIDENCE OF CARIOUS FIRST DECIDUOUS MOLARS IN WHITE AND NEGRO BOYS AND GIRLS STUDIED. (AGES 4 TO 14 YEARS INCLUSIVE)*

| TOTAL NUMBER OF BOYS EXAMINED: WHITE 681 NEGRO *435* | UPPER MOLARS | | | LOWER MOLARS | | | GRAND TOTAL |
|---|---|---|---|---|---|---|---|
| | RIGHT | LEFT | TOTAL | RIGHT | LEFT | TOTAL | |
| Number of first deciduous molars studied | 280<br>*201* | 288<br>*205* | 468<br>*406* | 256<br>*196* | 249<br>*204* | 505<br>*400* | 1073<br>*806* |
| Number of carious first deciduous molars | 88<br>*43* | 81<br>*45* | 169<br>*88* | 116<br>*88* | 122<br>*90* | 238<br>*178* | 407<br>*226* |
| Per cent of carious first deciduous molars | 31.4<br>*21.3* | 28.1<br>*22.0* | 36.1<br>*21.7* | 49.0<br>*44.9* | 49.0<br>*44.1* | 47.1<br>*44.5* | 37.9<br>*33.0* |

| TOTAL NUMBER OF GIRLS EXAMINED: WHITE 615 NEGRO *480* | UPPER MOLARS | | | LOWER MOLARS | | | GRAND TOTAL |
|---|---|---|---|---|---|---|---|
| | RIGHT | LEFT | TOTAL | RIGHT | LEFT | TOTAL | |
| Number of first deciduous molars studied | 208<br>*208* | 207<br>*214* | 415<br>*422* | 185<br>*199* | 196<br>*189* | 381<br>*388* | 796<br>*810* |
| Number of carious first deciduous molars | 84<br>*36* | 91<br>*46* | 175<br>*82* | 91<br>*66* | 109<br>*68* | 200<br>*134* | 375<br>*216* |
| Per cent of carious first deciduous molars | 40.4<br>*17.3* | 44.0<br>*16.8* | 42.2<br>*19.4* | 49.2<br>*33.2* | 55.6<br>*36.0* | 55.1<br>*34.5* | 47.1<br>*26.7* |

*The upper figure in each block refers to white children, the lower (italicized) figure to Negro children.

The lower first deciduous molars were those most frequently affected in the white boys and girls, the girls furnishing the higher percentage. In the Negro group also, the caries incidence of the lower first deciduous molars exceeded that of the upper. Here, however, the boys showed a greater proportion of such decayed teeth. It was difficult to surmise the reason for the increased caries incidence in the lower molars. The possibility that more lower molars than upper molars have deep cusp inclines, a factor claimed to be responsible for caries initiation, was fully considered but offered no solution. From a standpoint of anatomy all first deciduous molars (upper and lower) have shallow cusp inclines. A more plausible explanation was the apparently greater difficulty of keeping clean the lower interproximal spaces where caries of these teeth mostly originates. On the whole it seems that the lower interproximal areas are more retentive of food debris than the upper. In the face of this background there appear to be good reasons for a preponderance of lower carious molars.

There was a lack of similarity in caries frequency between the upper right and left sides in the white boys. In this group, however, the lower right and left molars showed an equal caries incidence. In the white girls a difference

was noticeable in the upper and lower molars of both sides. Bilateral caries which is supposed to be rather constant was only partly demonstrated in the white children. (Table III.)

The caries incidence in the Negro boys was nearly equal for upper and lower molars (right and left). In the Negro girls, however, a slight variation between the two upper sides and a more pronounced dissimilarity between the two lower sides was registered. Bilaterality, therefore, was rather consistent in the Negro males but inconsistent in the Negro females. It is probable that similar anatomic formations (Hyatt[7]) and similar environment (Leigh[8]) are not the only elements necessary to produce caries bilaterality. A fact which does not fit into the two theories just mentioned[7, 8] is that unilateral caries was noted in many of those studied.

TABLE III

GENERAL SUMMARY: INCIDENCE OF CARIOUS FIRST DECIDUOUS MOLARS IN WHITE AND NEGRO BOYS AND GIRLS STUDIED. (AGES 4 TO 14 YEARS INCLUSIVE)

| TOTAL NUMBER OF PUPILS EXAMINED (WHITE AND NEGRO GIRLS AND BOYS): 2,211 | UPPER MOLARS | | | LOWER MOLARS | | | GRAND TOTAL |
|---|---|---|---|---|---|---|---|
| | RIGHT | LEFT | TOTAL | RIGHT | LEFT | TOTAL | |
| Total number of first deciduous molars studied | 897 | 914 | 1811 | 836 | 838 | 1674 | 3485 |
| Total number of carious first deciduous molars | 251 | 263 | 514 | 361 | 389 | 750 | 1264 |
| Per cent of carious first deciduous molars | 29.1 | 28.8 | 28.4 | 43.2 | 46.1 | 45.3 | 35.7 |

## THE SECOND DECIDUOUS MOLARS

### ANALYSIS OF FINDINGS

Of the total number of 2037 second deciduous molars studied in the white boys and girls (Table IV), 1,040 (51 per cent) were carious. The Negro boys and girls showed a lower incidence of such decayed teeth. Of the total number of 1,845 second deciduous molars considered in them, 777 (42 per cent) were carious (Table IV). In both sexes and races filled teeth and those with fissures were not included in the count. Here again it was difficult to ascertain what influence promoted a lesser susceptibility to caries in the Negro children. It is not improbable that future investigations may throw light on this.

The white boys had a smaller percentage of carious second deciduous molars than the white girls, but the Negro boys showed a higher incidence than the Negro girls. Once again it was difficult to determine the relationship of sex to caries prevalence. The available data alone did not provide a sufficient scientific basis. If a comparison is instituted between the prevalence of carious second molars in the white and Negro boys, it will be learned that a slightly higher preponderance was detected in the former. The most important point was that the white girls showed a considerably greater proportion of such affected teeth than the Negro girls. There is no real explanation for the latter circumstance. It does seem clear, however, as previously intimated, that Negro children are less affected by caries regardless of geographical or climatic conditions, standards

of living, diet, etc. Sebelius[9] reported a lower caries index in a group of Negro children which he compared with an almost numerically equal group of white children in Tennessee.

TABLE IV

INCIDENCE OF CARIOUS SECOND DECIDUOUS MOLARS IN WHITE AND NEGRO BOYS AND GIRLS STUDIED. (AGES 4 TO 14 YEARS INCLUSIVE.)*

| TOTAL NUMBER OF BOYS EXAMINED: WHITE 681 NEGRO *435* | UPPER MOLARS | | | LOWER MOLARS | | | GRAND TOTAL |
|---|---|---|---|---|---|---|---|
| | RIGHT | LEFT | TOTAL | RIGHT | LEFT | TOTAL | |
| Number of second deciduous molars studied | 318<br>*237* | 319<br>*229* | 637<br>*466* | 261<br>*226* | 259<br>*221* | 520<br>*447* | 1157<br>*913* |
| Number of carious second deciduous molars | 146<br>*95* | 142<br>*87* | 288<br>*182* | 131<br>*111* | 130<br>*111* | 261<br>*222* | 549<br>*404* |
| Per cent of carious second deciduous molars | 45.9<br>*40.1* | 44.5<br>*37.9* | 45.2<br>*39.1* | 50.2<br>*49.1* | 50.2<br>*50.2* | 50.2<br>*49.7* | 47.5<br>*44.2* |

| TOTAL NUMBER OF GIRLS EXAMINED: WHITE 615 NEGRO *480* | UPPER MOLARS | | | LOWER MOLARS | | | GRAND TOTAL |
|---|---|---|---|---|---|---|---|
| | RIGHT | LEFT | TOTAL | RIGHT | LEFT | TOTAL | |
| Number of second deciduous molars studied | 263<br>*253* | 260<br>*253* | 523<br>*506* | 202<br>*220* | 205<br>*215* | 407<br>*435* | 930<br>*941* |
| Number of carious second deciduous molars | 135<br>*77* | 135<br>*96* | 270<br>*173* | 104<br>*99* | 117<br>*101* | 221<br>*200* | 491<br>*373* |
| Per cent of carious second deciduous molars | 51.3<br>*30.4* | 51.9<br>*37.9* | 51.6<br>*34.2* | 51.5<br>*45.0* | 57.1<br>*47.0* | 54.3<br>*46.0* | 52.8<br>*39.6* |

*The upper figure in each block refers to white children, the lower (italicized) figure to Negro children.

It was discovered that in both white and Negro children (male and female) the lower second deciduous molars were more often carious than their upper antagonists. This is really significant. The likelihood that more lower than upper second deciduous molars had deep occlusal fissures and thus were rendered more susceptible to the attacks of dental decay was fully scrutinized. Bossert's[10, 11] view that the steepness of the cusp inclines plays a prominent part in occlusal caries occurrence was not disregarded. However, it would be folly to assume that in all of these teeth caries was initiated at the bottom of the pit on their occlusal surfaces. As a matter of fact, the indications were that the interproximal regions with their food-retentive embrasures were more often involved and more frequently primarily attacked. The fundamental local cause of a higher prevalence of caries appeared to be the increased difficulty of keeping these areas clean.

Furthermore, it was observed that there were rather close similarities as well as variations (slight and marked) in caries frequency between the right and left sides (upper and lower) in both white and Negro children (male and female). This makes the task of judging bilaterality quite complicated. From the standpoint of the investigator, it is enough to say that this condition has justified its right to consideration. In many children it occurs to a noteworthy degree. What we still need to find out is the reason for its absence in many individuals of both sexes and of various races where unilateral caries prevails. (Table V.)

TABLE V

GENERAL SUMMARY: INCIDENCE OF CARIOUS SECOND DECIDUOUS MOLARS IN WHITE AND NEGRO BOYS AND GIRLS STUDIED. (AGES 4 TO 14 YEARS INCLUSIVE)

| TOTAL NUMBER OF PUPILS EXAMINED (WHITE AND NEGRO BOYS AND GIRLS): 2,211 | UPPER MOLARS | | | LOWER MOLARS | | | GRAND TOTAL |
|---|---|---|---|---|---|---|---|
| | RIGHT | LEFT | TOTAL | RIGHT | LEFT | TOTAL | |
| Total number of second deciduous molars studied | 1071 | 1061 | 2132 | 909 | 850 | 1759 | 3891 |
| Total number of carious second deciduous molars | 453 | 460 | 913 | 445 | 459 | 904 | 1817 |
| Per cent of carious second deciduous molars | 42.3 | 43.4 | 42.8 | 49.0 | 54.0 | 51.4 | 46.7 |

DISCUSSION

The first deciduous molars showed a lower incidence of caries than the second deciduous molars. We might suppose that the anatomical configuration of the first molars with their shallow cusp inclines renders them less subject to the accumulation and retention of food particles and, thus, to the deleterious effects of fermentation. The limits of this factor should be stressed, for in the main it appeared quite evident that the interproximal surfaces of these teeth serve much more frequently as regions for the initiation of caries (Tables VI, VII). The claim that a lower prevalence of fissures on the occlusal aspects of these teeth is responsible for a lower caries liability cannot be subscribed to.[12] The element which directly dominates the higher caries incidence in the second molars could not be determined. Very important, however, was the observation that food stagnation coupled with the greater difficulty of keeping the susceptible confines of these teeth clean seemed to play an important part in promoting this condition. All cases investigated suggested this hypothesis. It might also be added that in the 4- to 8-year age groups (on whom additional studies were carried out) the occlusal surfaces were more frequently attacked than those of the first deciduous molars. However, the cardinal point is the fact that

TABLE VI

INCIDENCE OF OCCLUSAL AND PROXIMAL CARIES OF FIRST DECIDUOUS MOLARS IN WHITE AND NEGRO BOYS AND GIRLS. (AGES 4 TO 7 YEARS INCLUSIVE)*

| AGES | NUMBER OF CHILDREN EXAMINED | | CARIES IN BOYS | | CARIES IN GIRLS | |
|---|---|---|---|---|---|---|
| | BOYS | GIRLS | OCCLUSAL | PROXIMAL | OCCLUSAL | PROXIMAL |
| 4 | 8 | 5 | 0 | 3 | 4 | 3 |
| | *6* | *2* | *0* | *0* | *0* | *0* |
| 5 | 41 | 36 | 13 | 28 | 4 | 23 |
| | *24* | *40* | *9* | *9* | *10* | *24* |
| 6 | 59 | 54 | 17 | 61 | 11 | 69 |
| | *29* | *37* | *2* | *23* | *6* | *20* |
| 7 | 43 | 47 | 15 | 36 | 21 | 44 |
| | *46* | *51* | *9* | *57* | 14 | *38* |
| Total | 151 | 142 | 45 | 128 | 40 | 139 |
| | *105* | *130* | *20* | *89* | *30* | *85* |

*The upper figure in each block refers to white children, the lower (italicized) figure to Negro children.

the incidence of interproximal caries of the second molars in these subjects exceeded that of occlusal caries. I regard this finding of great value (Tables VIII, IX).

TABLE VII

GENERAL SUMMARY: INCIDENCE OF OCCLUSAL AND PROXIMAL CARIES OF FIRST DECIDUOUS MOLARS IN 528 WHITE AND NEGRO CHILDREN. (AGES 4 TO 7 YEARS INCLUSIVE)*

| AGES AT LAST BIRTHDAY | NUMBER OF CHILDREN EXAMINED | | CARIES IN BOYS | | CARIES IN GIRLS | |
|---|---|---|---|---|---|---|
| | BOYS | GIRLS | OCCLUSAL | PROXIMAL | OCCLUSAL | PROXIMAL |
| 4 | 14 | 7 | 0 | 3 | 4 | 3 |
| 5 | 65 | 76 | 22 | 37 | 14 | 50 |
| 6 | 88 | 91 | 19 | 84 | 17 | 89 |
| 7 | 89 | 98 | 24 | 93 | 35 | 82 |
| Total | 256 | 272 | 65 | 217 | 70 | 224 |

*Proximal designates molars with mesial or distal caries or with incipient compound cavities involving only the very marginal ridge or end of their occlusal aspects. Molars with proximal caries involving larger areas than the marginal ridge or end of the occlusal surfaces were incorporated with those affected with occlusal caries.

I have entered into detail in the consideration of sex and race in their relationship to caries of these teeth because I feel that definite knowledge on this subject is important. Evidence of an inherent quality in the Negroes which renders them less susceptible to dental decay was contributed by this investigation, but I have not yet learned the nature of this protective element.

TABLE VIII

INCIDENCE OF OCCLUSAL AND PROXIMAL CARIES OF SECOND DECIDUOUS MOLARS IN WHITE AND NEGRO BOYS AND GIRLS. (AGES 4 TO 7 YEARS INCLUSIVE)*

| AGES | NUMBER OF CHILDREN EXAMINED | | CARIES IN BOYS | | CARIES IN GIRLS | |
|---|---|---|---|---|---|---|
| | BOYS | GIRLS | OCCLUSAL | PROXIMAL | OCCLUSAL | PROXIMAL |
| 4 | 8 | 5 | 8 | 0 | 6 | 1 |
| | *6* | *2* | *4* | *0* | *1* | *0* |
| 5 | 41 | 36 | 20 | 22 | 22 | 12 |
| | *24* | *40* | *19* | *9* | *21* | *26* |
| 6 | 59 | 54 | 41 | 49 | 30 | 47 |
| | *29* | *37* | *18* | *22* | *19* | *22* |
| 7 | 43 | 47 | 21 | 39 | 28 | 45 |
| | *46* | *51* | *29* | *58* | *24* | *48* |
| Total | 151 | 142 | 90 | 110 | 86 | 105 |
| | *105* | *130* | *70* | *89* | *65* | *96* |

*The upper figure in each block refers to white children, the lower (italicized) figure to Negro children.

TABLE IX

GENERAL SUMMARY: INCIDENCE OF OCCLUSAL AND PROXIMAL CARIES OF SECOND DECIDUOUS MOLARS IN THE 528 WHITE AND NEGRO CHILDREN (AGES 4 TO 7 YEARS INCLUSIVE)*

| AGES | NUMBER OF CHILDREN EXAMINED | | CARIES IN BOYS | | CARIES IN GIRLS | |
|---|---|---|---|---|---|---|
| | BOYS | GIRLS | OCCLUSAL | PROXIMAL | OCCLUSAL | PROXIMAL |
| 4 | 14 | 7 | 12 | 0 | 7 | 1 |
| 5 | 65 | 76 | 39 | 31 | 43 | 38 |
| 6 | 88 | 91 | 59 | 71 | 49 | 69 |
| 7 | 89 | 98 | 50 | 97 | 52 | 93 |
| Total | 256 | 272 | 160 | 199 | 151 | 201 |

*Proximal designates molars with mesial or distal caries or with incipient compound cavities involving only the very marginal ridge or end of their occlusal aspects. Molars with proximal caries involving larger areas than the marginal ridge or end of the occlusal surfaces were incorporated with those affected with occlusal caries.

TABLE X

PERFECT FIRST DECIDUOUS MOLARS (FREE FROM CARIES AND FISSURES AND WITH NO OPERATIVE WORK EVER PERFORMED ON THEM) IN WHITE AND NEGRO BOYS AND GIRLS. (AGES 4 TO 7 YEARS INCLUSIVE)*

| AGES AT LAST BIRTHDAY | WHITE AND NEGRO BOYS | | | | WHITE AND NEGRO GIRLS | | | |
|---|---|---|---|---|---|---|---|---|
| | NUMBER OF CHILDREN EXAMINED | NUMBER OF FIRST DECIDUOUS MOLARS EXAMINED | PERFECT FIRST DECIDUOUS MOLARS | | NUMBER OF CHILDREN EXAMINED | NUMBER OF FIRST DECIDUOUS MOLARS EXAMINED | PERFECT FIRST DECIDUOUS MOLARS | |
| | | | NO. | PER CENT | | | NO. | PER CENT |
| 4 | 8 | 31 | 27 | 87.1 | 5 | 20 | 10 | 50 |
| | *6* | *24* | *24* | *100* | *2* | *8* | *8* | *100* |
| 5 | 41 | 155 | 103 | 66.5 | 36 | 137 | 101 | 73.8 |
| | *24* | *95* | *76* | *80.0* | *40* | *158* | *116* | *73.4* |
| 6 | 59 | 227 | 131 | 57.7 | 54 | 211 | 107 | 50.7 |
| | *29* | *114* | *80* | *70.1* | *37* | *147* | *117* | *79.6* |
| 7 | 43 | 149 | 81 | 53.7 | 47 | 171 | 85 | 53.2 |
| | *46* | *180* | *105* | *58.3* | *51* | *197* | *136* | *69.0* |
| Total | 151 | 562 | 342 | 60.9 | 142 | 539 | 303 | 46.8 |
| | *105* | *413* | *285* | *69.0* | *130* | *510* | *377* | *73.9* |

*The upper figure in each block refers to white children, the lower (italicized) figure to Negro children.

Very significant was the disclosure that only 14 children (4 per cent) of the 340 comprising the 4-, 5-, and 6-year age groups had caries of all eight deciduous molars. Equally important was the discovery that in 104 (31 per cent) of the subjects comprising these brackets all eight deciduous molars were free from fillings and caries. These figures offer a sharp contrast to those of Martin.[6] Tables X and XI reveal the extent of freedom from caries and fissures of these teeth in the 528 children comprising the 4- to 7-year age groups. I believe that such grouping supplies a reliable index of the prevalence of perfect molars as it eliminates the factor of premature exfoliation. There can be no reasonable doubt that the missing molars in these subjects were lost through ex-

TABLE XI

PERFECT SECOND DECIDUOUS MOLARS (FREE FROM CARIES AND FISSURES AND WITH NO OPERATIVE WORK EVER PERFORMED ON THEM) IN WHITE AND NEGRO BOYS AND GIRLS. (AGES 4 TO 7 YEARS INCLUSIVE)*

| AGES AT LAST BIRTHDAY | WHITE AND NEGRO BOYS | | | | WHITE AND NEGRO GIRLS | | | |
|---|---|---|---|---|---|---|---|---|
| | NUMBER OF CHILDREN EXAMINED | NUMBER OF SECOND DECIDUOUS MOLARS EXAMINED | PERFECT SECOND DECIDUOUS MOLARS | | NUMBER OF CHILDREN EXAMINED | NUMBER OF SECOND DECIDUOUS MOLARS EXAMINED | PERFECT SECOND DECIDUOUS MOLARS | |
| | | | NO. | PER CENT | | | NO. | PER CENT |
| 4 | 8 | 32 | 18 | 56.2 | 5 | 19 | 10 | 52.6 |
| | *6* | *24* | *19* | *79.2* | *2* | *8* | *6* | *75* |
| 5 | 41 | 155 | 97 | 62.6 | 36 | 132 | 87 | 65.9 |
| | *24* | *95* | *62* | *65.3* | *40* | *157* | *101* | *64.3* |
| 6 | 59 | 219 | 108 | 49.3 | 54 | 203 | 105 | 51.7 |
| | *29* | *114* | *66* | *57.9* | *37* | *144* | *95* | *66.0* |
| 7 | 43 | 149 | 59 | 32.9 | 47 | 161 | 70 | 43.5 |
| | *46* | *175* | *82* | *46.9* | *51* | *191* | *102* | *53.4* |
| TOTAL | 151 | 555 | 282 | 50.8 | 142 | 515 | 272 | 52.8 |
| | *105* | *408* | *229* | *56.1* | *130* | *500* | *304* | *60.8* |

*The upper figure in each block refers to white children, the lower (italicized) figure to Negro children.

traction. Furthermore, an analytical study of the tables will show that the number of missing molars was relatively small. The Negro children showed a higher percentage of perfect deciduous molars.

SUMMARY

The incidence of caries of the deciduous molars was reported for a group of 2,211 white and Negro children of both sexes. Various social strata were represented. The caries incidence was higher in the white children than in the Negroes. The second deciduous molars were more frequently attacked by dental caries than the first. More lower than upper molars were found carious. Resistance to the initiation of caries was variously demonstrated in the males and females of the two races studied. Social status seemed to play no part in caries immunity as children of better economic levels showed a preponderance of affected molars.

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# THE DISTRIBUTION OF CARIOUS CAVITIES IN THE LOWER MOLARS OF CARIES-SUSCEPTIBLE AND CARIES-RESISTANT ALBINO RATS (RATTUS NORVEGICUS)

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IN 1937, Dr. Morris Steggerda of the Carnegie Institution of Washington suggested that we study the inheritance factor in dental caries of albino rats. A caries-susceptible and a caries-resistant line have been produced. More than 6,400 rats have been studied intensively, and 10,048 gross carious cavities have been identified in the lower molars. A graphic record has been kept of the site and growth of every cavity. The purpose of this paper is to report, with a minimum attempt to interpret observations, the distribution of these cavities with respect to: (1) the lower molar in which the cavity occurs, (2) the region of the molar affected, (3) whether the cavity is on the right or on the left jaw, and (4) whether it is found in a caries-susceptible or in a caries-resistant rat. It is hoped that such a report may be of value in the further search for causes of tooth decay.

The writers and their collaborators have shown that the size of food particles is a factor in producing cavities in rats' teeth,[1, 2, 9] that inheritance is an exceedingly important consideration,[3-8] that age appears to increase resistance,[9] that sex is not of consequence in causing rat caries,[10] but that use facilitates dental caries in rats.[8]

Hoppert and his co-workers devised a diet that would cause dental caries in rats.[1, 2] It consists, by weight, of 66 per cent coarsely ground hulled rice, 30 per cent whole milk powder, 3 per cent alfalfa leaf meal, and 1 per cent sodium chloride. The young rats have been introduced to this diet when 35 days old. Thereafter the teeth of the living animals have been examined every fourteen days, approximately, until at least one carious cavity has been identified positively. Phenotypic selection, progeny testing, and brother-sister inbreeding have been used to build one line susceptible to dental caries and an other line resistant to it.[6] When it was found that the coarse rice particles of the original diet were damaging the upper molars and thereby interfering with caries in the lower molars,[8] the rice was ground finer. This finer-textured ration was introduced with the thirteenth generation of resistant rats and the sixteenth generation of susceptibles.

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SYSTEM FOR LOCATING CARIOUS CAVITIES

The rat has three molar teeth on each lower jaw. The third molar is considerably smaller than the first and second, and appears to be degenerating in an evolutionary sense. Fig. 1 consists of much simplified diagrams of the right lower molars. The first molar has two deep transverse fissures whereas the second and third molars have one such fissure each. These fissures and the intervening cusps give the rats' molars a very rugged topography, quite unlike the corresponding teeth in man.

Fig. 1 provides a classification of the locations of carious cavities that agrees very well with our actual observations on the teeth. The black circles on the occlusal surfaces and the dark transverse bands between teeth are the sites at which decay may appear. For convenience in tabulating the cavities, the locations pictured in Fig. 1 are indicated by letters, *A* through *I*, and this plan is used in Tables I and II.

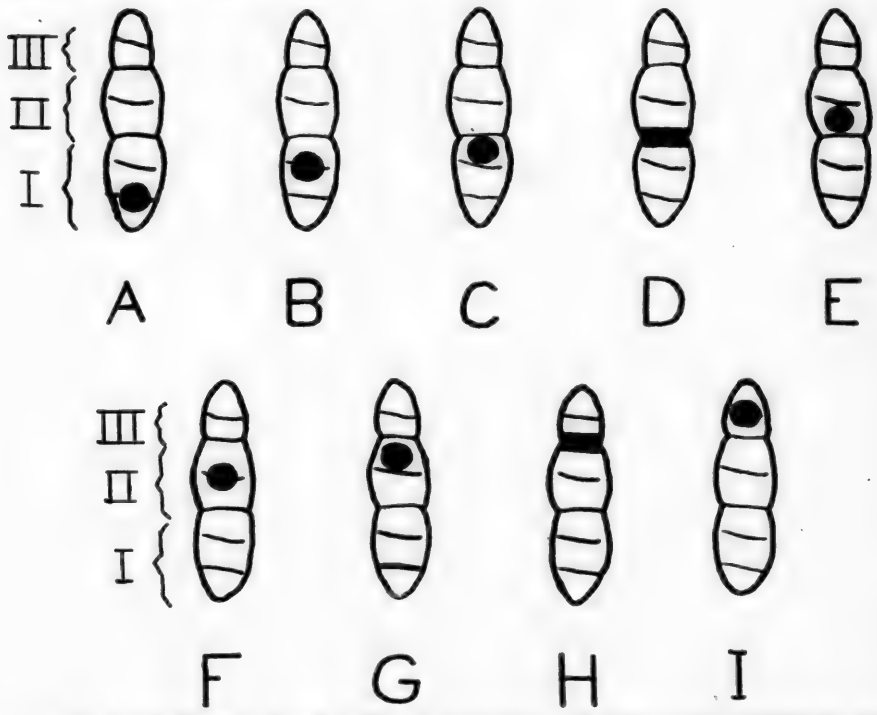


Fig. 1.—Locations of carious lesions in the lower molar teeth of the experimental rats. *I*, *II*, and *III* are the first, second, and third molars, respectively. *A* through *I* show in black circles or bars the positions of cavities.

Nearly all of the examinations of the teeth for cavities have been made by one of us (C.A.H.). The rats are examined approximately every two weeks. The animal is firmly held, ventral side up, in such a way as to avoid excessive movement due to struggling.* A nasal speculum is used to open the mouth and

*We wish to thank Mr. Earle D. Harrison, Superintendent of the Animal House of the Zoology Department, for care of the stock and skillful handling of the animals at the times of examination.

push away the cheek and tongue so that the molars can be clearly seen. The interior of the mouth is brightly illuminated with a shaded 100-watt lamp, 8 to 10 inches distant. Cavities can readily be detected with the unaided eye, and are usually revealed by more or less conspicuous impactions of food. A carious cavity may be identified positively at the first observation, or the first observation may not be decisive, in which case the next fortnightly examination may decide whether or not there is a cavity. Of course, the tiny beginning of a cavity cannot be detected in this manner, nor can the location of a hole be determined as accurately as by looking through a binocular microscope at a dissected jaw. There were probably some errors in assigning cavities to the nine sites used in Fig. 1. Such errors are doubtless distributed at random, so that they would tend to cancel each other. Moreover, adult rats' teeth are so easily seen that a cavity in one molar would probably not be assigned by mistake to an other.

### DISTRIBUTION IN CARIES-SUSCEPTIBLE RATS

Table I gives the distribution in percentages of 6,269 cavities found in all the rats of the caries-susceptible line of the second to nineteenth generations, inclusive. The first generation was, of course, the foundation stock from which both the susceptible and resistant strains were derived by selection and inbreeding. The table shows the distribution of cavities within each generation separately and for all eighteen generations combined in the two horizontal rows at the bottom. The vertical column at the extreme right shows the number of cavities recorded in each generation. The *R*, *L*, and *T* columns give the percentages of cavities on the right side, on the left side, and on both right and left combined, respectively, for each position on the teeth (*A*, *B*, *C*, etc.). The locus assigned to a cavity is the one at which it apparently first appeared. If at the first observation a whole molar had disappeared, the carious cavity was recorded as at the center of the occlusal surface of that molar, because that was the most probable point of origin of a process which eliminated the whole tooth.

There are striking differences in the frequencies of caries at the nine loci: at *A*, 0 per cent; at *B*, 21.7 per cent (1,362 cavities); at *C*, 4.0 per cent (248 cavities); at *D*, 10.6 per cent (665 cavities); at *E*, 0.7 per cent (43 cavities); at *F*, 38.7 per cent (2,424 cavities); at *G*, 14.9 per cent (933 cavities); at *H*, 9.5 per cent (593 cavities); at *I*, 0.02 per cent (1 cavity). The modal frequencies are at *B* (21.7 per cent) and at *F* (38.7 per cent), the central areas of the occlusal surfaces of the first and second molars. These high values may to some extent be due to the assignment to these loci of those cases in which the whole molar had disappeared by the first observation. There has been, in general, an increase in the frequency at both *B* and *F*. The frequencies at *D* and *H* have declined, perhaps as a result of the more accurate placement of cavities. There have been no marked changes in percentages at positions *C* and *E*. Beginning at the tenth generation, *G* shows a decline in incidence.

Particularly striking is the complete absence of caries at *A*, and its almost complete absence at *I*. By contrast with *A*, position *B* has a high incidence (21.7 per cent). *E* and *F* present an even greater contrast: 0.7 of one per cent

TABLE

PERCENTAGE DISTRIBUTION OF CARIOUS LESIONS

| GENERATION | LOCATIONS OF THE | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | | | B | | | C | | | D | | | E | | |
| | R | L | T | R | L | T | R | L | T | R | L | T | R | L | T |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2.7 | 1.2 | 3.8 | 18.9 | 20.7 | 39.5 | 0.3 | 0.6 | 0.9 |
| 3 | 0 | 0 | 0 | 0 | 0.2 | 0.2 | 1.0 | 1.4 | 2.4 | 19.5 | 16.9 | 36.4 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 4.7 | 1.0 | 5.7 | 13.3 | 17.3 | 30.6 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0.7 | .3 | 1.0 | 7.0 | 0 | 7.0 | 13.0 | 13.7 | 26.7 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0.9 | 1.1 | 2.0 | 9.9 | 3.1 | 13.0 | 6.8 | 9.9 | 16.7 | 0.3 | 0.3 | 0.6 |
| 7 | 0 | 0 | 0 | 0.6 | 0 | 0.6 | 4.7 | 3.1 | 7.8 | 6.8 | 13.7 | 20.5 | 1.6 | 1.9 | 3.4 |
| 8 | 0 | 0 | 0 | 11.6 | 7.7 | 19.3 | 5.2 | 5.6 | 10.7 | 2.6 | 6.4 | 9.0 | 0.4 | 0 | 0.4 |
| 9 | 0 | 0 | 0 | 17.8 | 10.8 | 28.5† | 5.6 | 4.7 | 10.3 | 0 | 1.9 | 1.9 | 0.5 | 0.5 | 0.9 |
| 10 | 0 | 0 | 0 | 13.6 | 9.2 | 22.7 | 4.4 | 3.1 | 7.5 | 2.0 | 2.7 | 4.8 | 1.4 | 0.7 | 2.0 |
| 11 | 0 | 0 | 0 | 20.7 | 8.3 | 29.0 | 2.4 | 2.4 | 4.7 | 1.2 | 2.4 | 3.6 | 0.6 | 0 | 0.6 |
| 12 | 0 | 0 | 0 | 15.3 | 3.3 | 18.6 | 1.8 | 1.5 | 3.3 | 1.8 | 2.3 | 4.1 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 20.1 | 4.8 | 24.9 | 0.5 | 0.7 | 1.1 | 1.1 | 1.6 | 2.7 | 0.7 | 0.7 | 1.4 |
| 14 | 0 | 0 | 0 | 28.8 | 6.6 | 35.4 | 0 | 0.7 | 0.7 | 0.2 | 0.7 | 0.9 | 0 | 0.2 | 0.2 |
| 15 | 0 | 0 | 0 | 35.2 | 11.9 | 47.1 | 0 | 0 | 0 | 0 | 0.4 | 0.4 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 29.2 | 10.9 | 40.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.4 | 0.8 | 1.2 |
| 17 | 0 | 0 | 0 | 30.3 | 9.9 | 40.2 | 0.6 | 0.8 | 1.4 | 0.2 | 0.2 | 0.3 | 0.2 | 0.8 | 1.0 |
| 18 | 0 | 0 | 0 | 28.9 | 7.5 | 36.4 | 0.5 | 1.0 | 1.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 | 18.9 | 1.5 | 20.5 | 0.4 | 0.8 | 1.2 | 0 | 0 | 0 | 0.4 | 0 | 0.4 |
| TOTALS | 0 | 0 | 0 | 16.4 | 5.3 | 21.7 | 2.5 | 1.5 | 4.0 | 4.8 | 5.8 | 10.6 | 0.3 | 0.4 | 0.7 |
| Total cavities | 0 | | | 1362 | | | 248 | | | 665 | | | 43 | | |

*The letters *A* through *I* indicate the locations of the cavities (Fig. 1). R = in the right lower molar
†All percentages in the table are at the nearest one-tenth of one per cent from the calculated value

TABLE

PERCENTAGE DISTRIBUTION OF CARIOUS LESIONS

| GENERATION | LOCATIONS OF THE | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | | | B | | | C | | | D | | | E | | |
| | R | L | T | R | L | T | R | L | T | R | L | T | R | L | T |
| 2 | 0 | 0 | 0 | 0.7 | 0 | 0.7 | 0 | 0.4 | 0.4 | 22.7 | 26.5 | 49.1 | 0 | 0 | 0 |
| 3† | 0 | 0 | 0 | 0.7 | 0.5 | 1.1 | 3.0 | 1.8 | 4.8 | 27.4 | 28.3 | 55.8 | 0.2 | 0.5 | 0.7 |
| 4‡ | 0 | 0 | 0 | 0.8 | 3.0 | 3.8 | 13.3 | 7.9 | 21.1 | 16.8 | 22.5 | 39.3 | 1.4 | 0.3 | 1.6 |
| 5 | 0 | 0 | 0 | 0.6 | 3.5 | 4.1 | 12.2 | 8.7 | 20.9 | 22.1 | 18.0 | 40.1 | 0.6 | 1.7 | 2.3 |
| 6 | 0.4 | 0 | 0.4 | 0.9 | 0.4 | 1.3 | 18.2 | 16.1 | 34.3 | 8.9 | 14.8 | 23.7 | 1.3 | 0.9 | 2.1 |
| 7 | 0 | 0 | 0 | 2.3 | 1.0 | 3.3 | 10.7 | 11.0 | 21.7 | 9.0 | 17.0 | 26.0 | 4.7 | 4.7 | 9.3 |
| 8 | 0 | 0 | 0 | 4.0 | 0.6 | 4.7 | 18.9 | 15.8 | 34.8 | 9.0 | 13.0 | 22.1 | 3.7 | 3.1 | 6.8 |
| 9 | 0 | 0 | 0 | 9.5 | 1.6 | 11.1 | 7.9 | 13.8 | 21.7 | 3.2 | 4.2 | 7.4 | 4.8 | 7.4 | 12.2 |
| 10 | 0 | 0 | 0 | 11.1 | 2.4 | 13.4 | 16.6 | 18.2 | 34.7 | 0.8 | 1.6 | 2.4 | 5.8 | 7.4 | 13.2 |
| 11 | 0 | 0 | 0 | 7.9 | 2.0 | 9.9 | 4.0 | 5.9 | 9.9 | 0.7 | 0.3 | 1.0 | 8.3 | 5.6 | 13.9 |
| 12 | 0 | 0 | 0 | 7.7 | 2.8 | 10.5 | 12.8 | 13.6 | 26.4 | 0.9 | 4.0 | 4.8 | 2.8 | 2.8 | 5.7 |
| 13 | 0 | 0 | 0 | 6.3 | 0 | 6.3 | 25.1 | 24.0 | 49.1 | 0.6 | 0.6 | 1.1 | 1.1 | 1.7 | 2.9 |
| 14 | 0 | 0 | 0 | 5.9 | 0.5 | 6.4 | 17.2 | 16.3 | 33.5 | 0 | 0 | 0 | 0.5 | 0.5 | 1.0 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | 16.0 | 18.0 | 34.0 | 0 | 2.0 | 2.0 | 4.0 | 6.0 | 10.0 |
| Totals | 0.03 | 0 | 0.03 | 4.4 | 1.4 | 5.8 | 11.7 | 11.1 | 22.8 | 10.0 | 12.5 | 22.5 | 2.8 | 2.9 | 5.7 |
| Total cavities | 1 | | | 219 | | | 861 | | | 852 | | | 215 | | |

All percentages in the table are at the nearest one-tenth of one per cent from the calculated value
*The letters *A* through *I* indicate the locations of the cavities (Fig. 1). R = in the right lower
†Includes same rats produced by matings between second and third generation parents.
‡Includes same rats produced by matings between third and fourth generation parents.

N THE LOWER MOLARS OF SUSCEPTIBLE RATS

| ARIOUS CAVITIES* | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F | | | G | | | H | | | I | | | TOTALS | | TOTAL CAVITIES |
| R | L | T | R | L | T | R | L | T | R | L | T | R | L | |
| 4.7 | 4.7 | 9.4 | 14.2 | 11.2 | 25.4 | 8.3 | 12.7 | 20.9 | 0 | 0 | 0 | 49.0 | 51.0 | 339 |
| 4.3 | 4.5 | 8.8 | 9.3 | 7.6 | 16.9 | 18.8 | 16.4 | 35.2 | 0 | 0 | 0 | 52.9 | 47.1 | 420 |
| 7.6 | 5.3 | 13.0 | 13.0 | 8.6 | 21.6 | 14.6 | 14.6 | 29.2 | 0 | 0 | 0 | 53.2 | 46.8 | 301 |
| 4.0 | 2.0 | 6.0 | 18.7 | 10.3 | 29.0 | 15.3 | 15.0 | 30.3 | 0 | 0 | 0 | 58.7 | 41.3 | 300 |
| 4.8 | 1.7 | 6.5 | 24.7 | 22.7 | 47.3 | 5.7 | 8.2 | 13.9 | 0 | 0 | 0 | 53.0 | 47.0 | 353 |
| 9.6 | 3.7 | 13.4 | 18.3 | 15.2 | 33.5 | 7.5 | 13.4 | 20.8 | 0 | 0 | 0 | 49.1 | 50.9 | 322 |
| 13.3 | 9.0 | 22.3 | 9.9 | 17.2 | 27.0 | 3.9 | 7.3 | 11.2 | 0 | 0 | 0 | 46.8 | 53.2 | 233 |
| 19.6 | 11.7 | 31.3 | 8.9 | 15.9 | 24.8 | 0 | 2.3 | 2.3 | 0 | 0 | 0 | 52.3 | 47.7 | 214 |
| 28.5 | 18.0 | 46.4 | 4.1 | 11.5 | 15.6 | 0 | 0.7 | 0.7 | 0.3 | 0 | 0.3 | 54.2 | 45.8 | 295 |
| 29.0 | 21.9 | 50.9 | 4.1 | 5.3 | 9.5 | 0 | 1.8 | 1.8 | 0 | 0 | 0 | 58.0 | 42.0 | 169 |
| 31.9 | 24.5 | 56.4 | 4.1 | 9.7 | 13.8 | 1.8 | 2.0 | 3.8 | 0 | 0 | 0 | 56.6 | 43.4 | 392 |
| 34.8 | 23.5 | 58.4 | 1.1 | 8.1 | 9.3 | 0.7 | 1.6 | 2.3 | 0 | 0 | 0 | 59.0 | 41.0 | 442 |
| 34.5 | 18.3 | 52.8 | 0.2 | 6.6 | 6.8 | 0 | 3.3 | 3.3 | 0 | 0 | 0 | 63.8 | 36.2 | 458 |
| 35.6 | 14.5 | 50.1 | 0 | 1.7 | 1.7 | 0 | 0.6 | 0.6 | 0 | 0 | 0 | 70.8 | 29.2 | 469 |
| 33.5 | 24.1 | 57.6 | 0 | 1.2 | 1.2 | 0 | 0 | 0 | 0 | 0 | 0 | 63.0 | 37.0 | 257 |
| 30.9 | 24.6 | 55.5 | 0.2 | 1.4 | 1.6 | 0 | 0 | 0 | 0 | 0 | 0 | 62.3 | 37.7 | 634 |
| 32.8 | 24.3 | 57.0 | 0.2 | 4.9 | 5.1 | 0 | 0 | 0 | 0 | 0 | 0 | 62.4 | 37.6 | 412 |
| 40.2 | 36.7 | 76.8 | 0 | 1.2 | 1.2 | 0 | 0 | 0 | 0 | 0 | 0 | 59.8 | 40.2 | 259 |
| 23.1 | 15.6 | 38.7 | 6.6 | 8.3 | 14.9 | 4.2 | 5.3 | 9.5 | 0.02 | 0 | 0.02 | 57.81 | 42.19 | |
| | 2424 | | | 933 | | | 593 | | | 1 | | | | 6269 |

= in the left lower molar. T = total cavities.

N THE LOWER MOLARS OF RESISTANT RATS

| ARIOUS CAVITIES* | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F | | | G | | | H | | | I | | | TOTALS | | TOTAL CAVITIES |
| R | L | T | R | L | T | R | L | T | R | L | T | R | L | |
| 0 | 1.7 | 1.7 | 4.9 | 3.8 | 8.7 | 19.9 | 19.5 | 39.4 | 0 | 0 | 0 | 48.1 | 51.9 | 287 |
| 1.4 | 1.1 | 2.5 | 3.4 | 4.8 | 8.2 | 16.3 | 10.7 | 27.0 | 0 | 0 | 0 | 52.4 | 47.6 | 441 |
| 1.9 | 1.9 | 3.8 | 7.1 | 4.9 | 11.9 | 7.9 | 10.3 | 18.2 | 0.3 | 0 | 0.3 | 49.3 | 50.7 | 369 |
| 0.6 | 1.7 | 2.3 | 5.2 | 5.2 | 10.5 | 9.9 | 9.9 | 19.8 | 0 | 0 | 0 | 51.2 | 48.8 | 172 |
| 2.6 | 3.4 | 5.9 | 8.5 | 11.4 | 19.9 | 3.8 | 6.8 | 10.6 | 1.7 | 0 | 1.7 | 46.2 | 53.8 | 236 |
| 1.7 | 2.7 | 4.3 | 13.7 | 13.7 | 27.3 | 3.3 | 4.3 | 7.7 | 0.3 | 0 | 0.3 | 45.7 | 54.3 | 300 |
| 0.6 | 1.2 | 1.9 | 11.5 | 12.7 | 24.2 | 2.5 | 3.1 | 5.6 | 0 | 0 | 0 | 50.3 | 49.7 | 322 |
| 8.5 | 6.9 | 15.3 | 12.2 | 14.8 | 27.0 | 2.7 | 1.6 | 4.2 | 1.1 | 0 | 1.1 | 49.7 | 50.3 | 189 |
| 7.6 | 3.2 | 10.8 | 10.8 | 13.2 | 24.0 | 0.8 | 0.8 | 1.6 | 0 | 0 | 0 | 53.4 | 46.6 | 380 |
| 21.8 | 7.3 | 29.0 | 15.8 | 20.1 | 36.0 | 0.3 | 0 | 0.3 | 0 | 0 | 0 | 58.7 | 41.3 | 303 |
| 16.2 | 1.7 | 17.9 | 17.3 | 16.2 | 33.5 | 0 | 0.6 | 0.6 | 0.3 | 0.3 | 0.6 | 58.0 | 42.0 | 352 |
| 14.9 | 1.7 | 16.6 | 13.1 | 10.9 | 24.0 | 0 | 0 | 0 | 0 | 0 | 0 | 61.1 | 38.9 | 175 |
| 18.2 | 4.9 | 23.2 | 12.3 | 21.7 | 34.0 | 0 | 0 | 0 | 1.0 | 1.0 | 2.0 | 55.2 | 44.8 | 50 |
| 16.0 | 2.0 | 18.0 | 18.0 | 18.0 | 36.0 | 0 | 0 | 0 | 0 | 0 | 0 | 54.0 | 46.0 | 203 |
| 7.0 | 2.8 | 9.9 | 10.4 | 11.5 | 21.9 | 5.6 | 5.4 | 11.0 | 0.3 | 0.1 | 0.4 | 52.18 | 47.82 | |
| | 373 | | | 828 | | | 416 | | | 14 | | | | 3779 |

olar. L = in the left lower molar. T = total cavities.

at *E*, 38.7 per cent at *F*. Such contrasts must be correlated with at least some of the causes of dental caries.

Beginning with the sixteenth generation, the rice of the cariogenic ration was ground finer than previously to avoid mechanical injury to the upper molars. There was no great change in the frequency of caries at any site as a consequence of this modification of diet.

A very interesting fact is that Table I shows significantly more cavities on the right jaw (57.81 per cent) than on the left (42.19 per cent). If the cavities were distributed at random, half of them ($\frac{1}{2} \times 6{,}269$) should be on the right side and half on the left. The standard deviation of such a distribution would be 39.6.* The deviation of the observed distribution from the expected random distribution is 3,624 (observed cavities on the right side), minus 3,134.5 ($\frac{1}{2} \times 6{,}269$), which is 489.5. This deviation is 12.4 times the standard deviation of the normal distribution and would occur by chance once in millions of times. Thus our result is probably not due to chance. Table I shows that in 15 of the 18 generations there have been more cavities on the right than on the left side, and that there has been a tendency for the right side to increase its lead over the left.

### DISTRIBUTION IN CARIES-RESISTANT RATS

Table II gives the percentage distribution of 3,779 carious lesions in the lower molars of resistant rats from the second to the fifteenth generations, inclusive. The organization of this table is the same as for Table I. As far as possible (except for positions *D* and *H*) the site of the cavity is indicated at the location the cavity first appeared. When a small cavity is first found at position *D* or *H*, it is difficult to tell in which molar of the pair it is located; a subsequent observation, when the cavity has enlarged, usually settles the point. In a caries-resistant animal a hole very rarely if ever increases so rapidly as to demolish the whole crown before the first observation.

The nine locations show large differences in the frequencies of caries. These frequencies are: at site *A*, 0.03 per cent (1 cavity); at *B*, 5.8 per cent (219 cavities); at *C*, 22.8 per cent (861 cavities); at *D*, 22.5 per cent (852 cavities); at *E*, 5.7 per cent (215 cavities); at *F*, 9.9 per cent (373 cavities); at *G*, 21.9 per cent (828 cavities); at *H*, 11.0 per cent (416 cavities); and at *I*, 0.4 per cent (14 cavities). The modes are at *C*, *D*, and *G*, which are the posterior region of the first molar, the contact between the first and second molars, and the posterior part of the second molar, respectively. Thus, there are not the high concentrations at *B* and *F* which are found in the susceptibles. There is a decrease with advancing generations in the frequencies at *D* and *H*. *D* and *H* are nondescript positions—neither specifies which of the two molars involved harbors the cavity. As the experiment proceeded, more skill may have been developed in locating cavities accurately; but the more important factor probably is that, as the resistant strain increased in resistance, individual rats were examined over a longer period of time, so there was greater opportunity for a

*Standard deviation $= \sqrt{npq}$ where $n = 6{,}269$, and $p = q = \frac{1}{2}$ = probability that any one cavity will be on the right or on the left side.

cavity to enlarge and show clearly to which molar it belonged. This explanation is supported by the fact that the frequencies under *D* in general decline and those for *C* increase with passing generations. It would seem that, likewise, *H* loses in favor of *G*. The change from the "coarse" rice to the "fine" rice-containing diet at the thirteenth generation made no consistent difference in the distribution of cavities.

As in the susceptible line, there are significantly more cavities on the right jaw (52.18 per cent) than on the left (47.82 per cent), though in these resistant animals the difference between right and left is not as great. A normal (random) distribution for 3,779 cavities would have a standard deviation of 30.7. The observed number of right-jawed cavities (1,972) deviates from 1,889.5 ($\frac{1}{2} \times 3{,}779$) by 82.5, which is 2.69 times as large as the standard deviation, 30.7. Such a deviation in such a number of items would occur by chance about once in 278 times. This excess in the number of right-sided cavities is statistically significant. In 9 of the 14 generations included in Table II, the carious lesions on the right side exceed those on the left.

The susceptible strain has a significantly higher frequency of cavities on the right jaw (57.81 ± .42 per cent) than the resistant strain (52.18 ± .55 per cent). The difference (5.63 ± .69 per cent) is 8.16 as large as its probable error. The odds against getting such a difference by chance are millions to one.

It is worth mentioning that whereas an error may be made in assigning a cavity to one position on a tooth, there is no possibility of observational error in determining whether it is on the right side or on the left.

## DISCUSSION

We have not demonstrated the cause (or causes) for the high dextral incidence of dental caries in these rats, but an interesting hypothesis is suggested. We have shown[8] that use of a molar tooth for mastication in a caries-susceptible rat facilitates the carious process in that tooth. Apparently pressure on the rice particles in the cariogenic diet accelerates the development of cavities. If a rat is a "right-handed" chewer, the lower right molars would be affected in this manner more than the ones on the left side and would develop carious lesions sooner. Our observations cease as soon as one cavity has been definitely observed. The result would be that if a majority of our rats were right handed in chewing, the majority of lesions should be on the right side. Perhaps it would be pertinent to investigate variations in the mechanics of mastication in our animals.

The main objective of our research since 1937 has been to determine the importance of the inheritance factor. The resistance of an animal has been measured by the days elapsing on the cariogenic diet until the appearance of the first cavity. Sometimes, of course, several cavities appear simultaneously. The present paper therefore deals with the distribution of earlier cavities—not all cavities. The influence of one lesion on the appearance of later ones has thus been minimized.

In more than 6,400 rats we have never observed a carious lesion in the incisors, and one of our graduate students, R. F. Keller, is confirming a previous impression that caries is much rarer in the upper molars than in the lowers.

This paper has not proved the potency of any new factor in dental caries of the rat, but it is hoped that the facts recorded may be of future use in this quest. In previous papers we have shown that heredity, texture of the food, use, and, to some extent, age, are of importance. Lactobacilli are doubtless one of the chief links in the chain of causation. Among the other factors that should be investigated are: impaction of food in the deep crevices of the molars, susceptibility to fracturing (minor chipping and major breaks), cleansing movements of the cheek and tongue, the amount and chemical nature of the saliva, relation to the openings of the salivary ducts, the mechanics of mastication in the rat, etc.

One generalization of great importance has emerged—dental caries in rats is not due to any one cause but is the resultant of a number of factors and processes.

## SUMMARY

1. Some areas on the occlusal surfaces of the rat's lower molar teeth are more susceptible to caries than others.

2. The right lower molars develop more carious cavities than the left.

3. The excess of caries in the right lower molars of the susceptible strain is greater than the excess in the resistant stock.

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# CORRELATION OF SALIVA ANALYSES WITH DENTAL EXAMINATIONS OF 574 FRESHMEN AT OREGON STATE COLLEGE

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DURING the winter and spring quarters of the 1946-1947 school year, dental examinations* were made on those freshman students who, according to the registrar's office, were residents of Oregon and who had not been in the armed services. Nutrition histories† were taken and samples of saliva were collected for bacteriological and chemical examinations.

The relationship between the characteristics of the saliva and dental caries experience has been studied by a number of investigators. Some relationships, for example the correlation between lactobacillus counts and dental caries, have been reported to be so definitely indicative of carious activity as to recommend their use as diagnostic tests. The results of other tests, however, have been so conflicting as to suggest the need for further studies before their real value can be established. The present study offered an opportunity to conduct chemical and bacteriological tests on the saliva samples of 574 students. An attempt was made to include not only those tests whose values have been confirmed but also some of the tests which have not been studied extensively. The analyses included in this study were made with the idea of studying their relationship to dental caries incidence as well as their relationship to each other. The results of these tests were then compared with the DMF surfaces which represent the cumulative caries experience of the individual.

## PROCEDURE

When students reported for their appointments, they were brought first to the laboratory where a paraffin‡-stimulated sample of saliva was collected in a sterile 50 ml. graduate cylinder. The rate of flow, in terms of milliliters per minute, was calculated by noting the volume collected in a timed 5-minute period. The determinations were made immediately after the collection of the saliva and were done in the following order: pH, starch hydrolyzing time, buffer capacity, ammonia nitrogen, and finally the three dilutions for the lactobacillus counts were plated and placed in the incubator. Appointments were scheduled

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†In manuscript.

‡Paraffin sticks wrapped similarly to chewing gum were furnished through the courtesy of Dr. L. S. Fosdick, Northwestern University Dental School.

approximately every half hour, from 8:00 A.M. to 11:00 A.M. and from 1:00 P.M. to 3:30 P.M.

The methods used were as follows:

1. *pH.* The pH of the stimulated saliva was determined on the Beckman Model G pH meter.

2. *Starch Hydrolyzing Time.* These determinations were made according to the method of Turner and Crane.[1] It was found necessary to dilute the saliva 1:10 in distilled water in order to get a satisfactory end-point reading. One ml. of diluted saliva was added to 1 ml. of 1 per cent soluble starch solution and a stop watch started. Four drops of the saliva-starch mixture were added to 1 drop of potassium triiodide solution in a spot plate at 15-second intervals for the first minute and at 30-second intervals thereafter until the blue color disappeared and a medium red-brown appeared. The time necessary for the appearance of the end point was noted.

3. *Buffering Capacity.* The buffering capacity was calculated according to the method of Deakins, Cheyne, Bibby, and Van Kesteren.[2] In this method, a 2-ml. aliquot of saliva was titrated to a methyl red end point with 0.01N HCl and another 2-ml. aliquot was titrated to a phenolphthalein end point with 0.01N NaOH. The amount of 0.01N acid plus the amount of 0.01N base required for adjusting 1 ml. to the respective end points was multiplied by the rate of flow and divided by 4 (the approximate number of pH units between the methyl red and phenolphthalein end points). The results for the buffering capacity of the saliva, therefore, were expressed as a value which took into consideration the rate of flow.

4. *Ammonia Nitrogen.* The ammonia nitrogen was determined by the formol titration method as described by Arnold and McClure.[3] One ml. of saliva and 1 ml. of 10 per cent potassium oxalate were mixed in a test tube. Using phenolphthalein as an indicator, the mixture was titrated to a faint pink with 0.002N NaOH. One ml. of 30 to 40 per cent commercial formalin that had been titrated to a faint pink with 0.2N NaOH, using phenolphthalein as an indicator, was added. The mixture was again titrated to a faint pink with 0.002N NaOH. This second titration value is a measure of the ammonia nitrogen. One ml. of 0.002N NaOH is equivalent to 0.028 mg. ammonia nitrogen.

5. *Lactobacillus Counts.* The lactobacillus counts were made according to the method of Hadley.[4] Saliva was diluted 1:5, 1:20, and 1:100 in prescription bottles. These were shaken 50 times and 0.1 ml. of each was spread with a sterile bent glass rod over the surface of a plate of tomato juice-peptone agar (pH 5.0). Plates were incubated 96 hours, at the end of which time the colonies of lactobacilli were counted with the aid of a colony counter. The count was expressed as the number of lactobacilli per ml. of saliva.

## RESULTS

The means and ranges of the five tests grouped according to the number of DMF surfaces are presented in Table I.

Starch hydrolyzing time was recorded as the number of seconds required to reach the red-brown end point. Since it was rarely possible to obtain a readable end point using undiluted saliva, it was found best to dilute most of the samples 1:10. All results, therefore, were reported on a 1:10 dilution basis. The mean of the lactobacillus counts was omitted as it was thought that a better perspective of the distribution could be shown in Fig. 1.

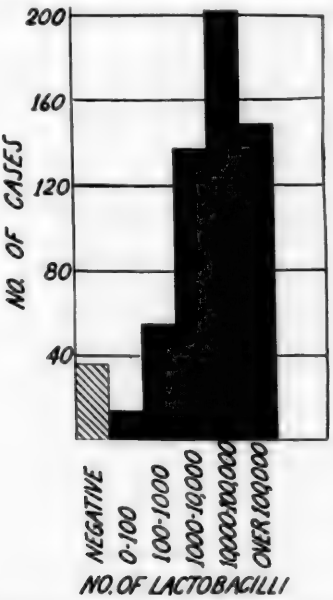


Fig. 1.—Frequency distribution of numbers of lactobacilli.

Correlation coefficients were calculated using the original data for each subject to see if any of these salivary analyses were related significantly to past dental caries experience. The statistical procedures followed were those of Snedecor.[6] In the final expression of a correlation coefficient, an answer of +1.0 indicates a perfect direct correlation. In other words, in every case, when the results of one particular test increase, the results of the other test increase proportionately. Conversely, an answer of –1.0 indicates a perfect inverse correlation. In this case, if all results of one particular test go up, the results of the other decrease proportionately. The degree to which two tests correlate is expressed as a fraction between +1.0 and –1.0. The larger this fraction is, the greater is the possibility that the correlation is statistically significant. In order to test the significance of a correlation coefficient, the "t" test[6] was applied. The basis of this test is the null hypothesis that the drawing is made from a population with zero correlation. The levels of significance vary with the size of the sample. When the sample size reaches the 500 level, the minimum level of significance is 0.088. Coefficient of correlation values above 0.115 are highly significant, values between 0.088 and 0.115 are significant, and values below 0.088 are not statistically significant.

TABLE I

RESULTS OF FIVE SALIVARY ANALYSES AND DMF EXPERIENCES OF 574 FRESHMAN STUDENTS

| DMF SURFACES | NO. OF CASES | STARCH HYDROLYZING TIME | | pH* | | BUFFER CAPACITY | | AMMONIA NITROGEN | | LACTOBACILLUS COUNT |
|---|---|---|---|---|---|---|---|---|---|---|
| | | MEAN (SECONDS) | RANGE (SECONDS) | MEAN | RANGE | MEAN | RANGE | MEAN MG./100 ML. | RANGE MG./100 ML. | RANGE |
| 0- 5 | 20 | 75 | 30-240 | 7.22 | 6.20-7.75 | 1.18 | 0.25-2.25 | 1.63 | 0.00-7.50 | 0-85,000 |
| 6-10 | 27 | 130 | 15-1200 | 7.48 | 7.18-7.93 | 1.22 | 0.26-2.51 | 1.97 | 0.00-5.05 | 0-195,000 |
| 11-15 | 39 | 88 | 6-900 | 7.47 | 7.21-7.77 | 1.14 | 0.38-2.74 | 1.79 | 0.00-6.02 | 0-too thick |
| 16-20 | 45 | 91 | 30-900 | 7.47 | 7.05-7.82 | 1.05 | 0.20-2.64 | 1.71 | 0.00-6.27 | 0-too thick |
| 21-25 | 62 | 114 | 15-1350 | 7.48 | 7.15-7.87 | 1.28 | 0.35-4.29 | 2.04 | 0.00-14.46 | 0-too thick |
| 26-30 | 65 | 114 | 18-1350 | 7.44 | 6.95-7.92 | 1.01 | 0.27-2.76 | 2.23 | 0.00-10.18 | 0-too thick |
| 31-35 | 64 | 69 | 30-450 | 7.38 | 6.92-8.20 | 1.04 | 0.10-2.92 | 2.03 | 0.00-9.51 | 0-too thick |
| 36-40 | 59 | 91 | 15-750 | 7.43 | 7.01-7.82 | 1.08 | 0.06-2.73 | 1.88 | 0.00-8.51 | 0-too thick |
| 41-45 | 50 | 135 | 15-1800 | 7.43 | 6.98-7.82 | 1.09 | 0.29-2.32 | 1.94 | 0.00-8.25 | 0-too thick |
| 46-50 | 47 | 100 | 15-1200 | 7.38 | 6.78-7.78 | 1.08 | 0.18-2.31 | 1.91 | 0.00-10.68 | 0-too thick |
| 51-55 | 36 | 75 | 30-450 | 7.41 | 6.93-7.71 | 0.89 | 0.10-1.82 | 2.23 | 0.00-8.58 | 200-too thick |
| 56-60 | 26 | 57 | 30-165 | 7.40 | 6.93-7.79 | 1.09 | 0.37-2.59 | 1.82 | 0.00-9.94 | 750-too thick |
| 61-65 | 11 | 68 | 30-180 | 7.38 | 6.97-7.63 | 0.82 | 0.25-1.54 | 1.93 | 0.11-3.34 | 6,800-307,000 |
| 66-70 | 9 | 73 | 45-120 | 7.29 | 6.97-7.60 | 0.71 | 0.21-1.68 | 1.52 | 0.00-3.63 | 850-too thick |
| 71-75 | 6 | 60 | 45-90 | 7.36 | 7.13-7.63 | 0.98 | 0.53-1.92 | 2.15 | 0.63-4.19 | 150-720,000 |
| 76-80 | 4 | 64 | 30-120 | 7.20 | 6.92-7.42 | 0.79 | 0.61-1.00 | 2.37 | 0.00-3.61 | 29,600-too thick |
| 81-85 | 2 | 32 | 18-45 | 7.37 | 7.21-7.62 | 1.00 | 0.25-1.75 | 1.26 | 0.77-1.74 | all plates too thick |
| All Cases | 572 | 95 | 6-1800 | 7.42 | 6.20-8.20 | 1.08 | 0.06-4.29 | 1.96 | 0.00-14.46 | |

*The means for pH were determined by converting each pH value to acid activity. The mean of the acid activity was then calculated and converted into pH by referring to the table in Bridges and Mattice.[5]

The results of the salivary analyses were correlated individually with the clinical findings as expressed by the DMF surfaces. Then the "t" test was applied as described above to these coefficients to determine whether or not they were statistically significant. These correlations and their statistical significance are shown in Table II.

TABLE II

CORRELATIONS BETWEEN SALIVARY ANALYSES AND CLINICAL FINDINGS AS EXPRESSED BY DMF SURFACES*

| ITEMS CORRELATED | CORRELATION COEFFICIENT | STATISTICAL SIGNIFICANCE |
|---|---|---|
| DMFs and starch hydrolyzing time | −.460 | Highly significant |
| DMFs and lactobacillus count | +.245 | Highly significant |
| DMFs and pH | +.196 | Highly significant |
| DMFs and buffer capacity | −.123 | Highly significant |
| DMFs and ammonia nitrogen | +.036 | Not significant |

*By DMF surfaces is meant the total number of tooth surfaces that are decayed, missing, or filled.

It will be observed from the above table that there was a marked negative correlation between DMF surfaces and starch hydrolyzing time. That is to say, in those cases with a high number of DMF surfaces, there was also a rapid hydrolysis of starch by the saliva. There was a marked positive correlation between DMF surfaces and the numbers of lactobacilli in the saliva. Both of these correlations were highly significant. The correlation between DMF surfaces and pH was also highly significant. It will be noted, however, from the Table II that there was a highly significant negative correlation between buffer capacity and DMF surfaces. This indicates that in those cases with saliva of a high buffer capacity, the number of DMF surfaces was low. The only correlation value which was not found to be statistically significant was the one between DMFs and ammonia nitrogen.

After correlating the salivary analyses with the clinical findings, it was decided to see if there were any significant interrelationships among the tests themselves. These intercorrelations together with their statistical significance are shown in Table III.

TABLE III

CORRELATIONS AMONG THE SALIVARY ANALYSES AND THEIR STATISTICAL SIGNIFICANCE

| ITEMS CORRELATED | CORRELATION COEFFICIENT | STATISTICAL SIGNIFICANCE |
|---|---|---|
| pH and buffer capacity | +.252 | Highly significant |
| Lactobacillus count and buffer capacity | −.109 | Significant |
| Buffer capacity and starch hydrolyzing time | +.102 | Significant |
| Ammonia nitrogen and pH | +.098 | Significant |
| Ammonia nitrogen and buffer capacity | −.085 | Not significant |
| Starch hydrolyzing time and lactobacillus count | −.082 | Not significant |
| Starch hydrolyzing time and pH | +.063 | Not significant |
| pH and lactobacillus count | +.032 | Not significant |
| Starch hydrolyzing time and ammonia nitrogen | −.028 | Not significant |
| Lactobacillus count and ammonia nitrogen | −.020 | Not significant |

As shown in Table III, the correlation between pH and buffer capacity was highly significant. In other words, when the saliva had a high pH, the buffer capacity was also high. The inverse correlation between the numbers

of lactobacilli and the buffer capacity was also significant. Those salivas that had high lactobacilli counts had low buffer capacities. There also was a significant positive correlation between buffer capacity and starch hydrolyzing time. The correlation coefficients indicated that saliva specimens high in ammonia nitrogen were also high in pH.

## DISCUSSION

The rapidity with which the saliva hydrolyzes starch is a measure of its amylase activity. The relationship between this activity and caries experience has been studied by a number of investigators. Florestano, Faber, and James[7] found a positive relationship between the amylase activity and the incidence of caries. Turner and Crane[1] in a similar study also found a positive relationship between starch hydrolyzing time and the activity of caries. Our findings corroborate those of the above investigators; that is, we found a marked correlation between past caries experience and starch hydrolyzing time.

In this study the most highly significant correlation was found between caries experience, as expressed by DMF surfaces, and starch hydrolyzing time. This would seem to be logical if one gives credence to the acid theory of caries production. Bergeim and Barnfield,[8] on the other hand, did not find any correlation between amylase activity and caries activity.

Even earlier than Miller[9] who, in 1889, advanced the theory that the acid present in carious mouths was produced by microorganisms such a mechanism had been suggested. Since that time much work has been done on the relationship between the numbers of lactobacilli in the saliva and the extent of dental caries. Bunting and Palmerlee[10] found a close correlation between the presence of lactobacilli and active caries. Jay and Voorhees[11] were able to isolate lactobacilli consistently from carious mouths and, at the same time, were unable to isolate them from caries-free mouths. The Michigan group continued intensive investigations and the conclusion was reached that *Lactobacillus acidophilus* could be a specific causative organism for dental caries. This theory gained wide acceptance but was as strongly opposed by those upholding the theory of proteolytic bacteria as the causative agent in dental caries (Gottlieb[12]). At the present time, however, most investigators agree that there is some connection between the acid-producing members of the oral flora and caries initiation. The relationship between lactobacilli and caries incidence is accepted widely enough now to be used in many laboratories as a diagnostic aid. Jay, Crowley, Hadley, and Bunting,[13] as early as 1933, evolved a diagnostic procedure employing lactobacillus counts.

Our results also show a highly significant correlation between DMF surfaces and lactobacillus counts. The correlation might have been higher if the time of day for the collection of saliva could have been standardized. More than one count on each student, too, would have given a more accurate picture, since the count is known to vary from time to time. In spite of these drawbacks the data show high counts corresponding to high DMF surfaces.

The problem of pH and dental caries is one that has been investigated widely and with varying results. Hubbell[14] and Karshan[15] reported no correlation between pH of saliva and dental caries. Campaigne[16] also reported lack of

correlation using the antimony electrode. Stephan,[17] using colorimetric methods, was unable to show extreme variation in salivary pH. Swerdlove,[18] using Stephan's procedures, corroborated his results. Fosdick and Campaigne,[19] however, did note a difference in pH between carious and noncarious areas within the mouth.

Our determinations of pH were done on stimulated saliva that was not collected under oil. Although it was realized that error was introduced because of the loss of carbon dioxide, it was thought that relative results could be obtained by using a constant collection time of 5 minutes. This was soon found to be impossible as some subjects needed up to 20 minutes in which to collect the minimum amount of saliva needed for analysis. The results show a positive correlation with DMF surfaces, whereas one might logically expect an inverse correlation. No explanation for this can be advanced at this time.

Marshall[20] in 1915 first observed a relationship between the buffer capacity of saliva and caries experience. His work was assailed vigorously by Gies and Shepard.[21] Other investigators, however, have shown a correlation between the extent of caries and buffering capacity. Hubbell,[14] Karshan,[15] and Fosdick, Campaigne, and Fancher[22] have all found that the buffering capacity of saliva from caries-immune people is higher than that from caries-susceptible people. Our results seem to bear out these findings. In the study here reported, a significant inverse correlation between buffer capacity and DMF surfaces was found.

The amount of ammonia nitrogen present in saliva has received considerable attention. Grove and Grove[23] were able to demonstrate a difference in the ammonia-nitrogen content of saliva between caries-immune and caries-susceptible subjects. White and Bunting[24] reported no correlation in similar studies. Recently Kesel, O'Donnell, Kirch, and Wach[25] have been able to show a significant reduction of the numbers of lactobacilli by incorporating dibasic ammonium phosphate in a mouthwash and dentifrice.

## SUMMARY

Analyses were done at Oregon State College on saliva samples from 574 freshman students who, according to the registrar's office, were residents of Oregon and who had not been in the armed services. The five tests that were done are: pH, starch hydrolyzing time, buffer capacity, ammonia nitrogen, and lactobacillus plate counts. Saliva was collected by paraffin stimulation and all tests were performed immediately after collection.

Correlation coefficients were calculated between the five tests and clinical findings as expressed by DMF surfaces. Highly significant correlations were found between DMF surfaces and starch hydrolyzing time and between DMF surfaces and lactobacillus counts.

There was also a significant positive correlation between DMF surfaces and pH, as well as a significant inverse correlation between DMF surfaces and buffer capacity. There was no significant correlation between DMF surfaces and ammonia nitrogen.

Intercorrelations among the various analyses were calculated, using each of ten possible combinations. There was a highly significant correlation co-

efficient between pH and buffer capacity. There was a significant inverse correlation between lactobacillus plate counts and buffer capacity; also, there were significant positive correlations between buffer capacity and starch hydrolyzing time and between ammonia nitrogen and pH.

Appreciation is expressed to Dr. Margaret L. Fincke, Head of the Department of Foods and Nutrition at Oregon State College; to Dean Harold J. Noyes, Dental School, University of Oregon; to Dr. O. T. Wherry, member of the Oregon State Board of Health; and to Dr. E. R. Abbett, representative of the Oregon State Dental Association, for their helpful suggestions and criticisms.

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# STATISTICAL INTERPRETATION OF SALIVARY ANALYSES ON 555 SCHOOL CHILDREN IN TWO GEOGRAPHIC REGIONS IN OREGON

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A STUDY was made on specimens of saliva collected from native-born and native-reared school children who were 14, 15, and 16 years old in four counties of two regions of Oregon.* This paper is a report of the findings from the examination of the saliva of 555 children, the number of subjects on whom all of the salivary data were obtainable.

The facilities for the salivary analyses as well as those for the dental examinations were housed in a large U. S. Public Health Service laboratory trailer which was moved from county to county. One end of the trailer was used for the salivary analyses, while the dental equipment was installed in the other end. A crew of six people worked regularly in the trailer; a dentist, an x-ray technician, a bacteriologist, a laboratory assistant, and two people to keep laboratory glassware clean and to sterilize equipment. The trailer was supplied with water and electric power by direct connections to the school building which was used as headquarters.

## METHODS

The analyses made on the saliva were: pH, buffer capacity, lactobacillus counts, amylase activity as measured by starch hydrolyzing time, ammonia nitrogen, and the acid producing power of the oral flora as measured by the Snyder test.[2] The first five are the same analyses as were made on the freshman students at Oregon State College.[2]

The Snyder test was done as follows: One-tenth of a milliliter of a 1:10 dilution of saliva was inoculated into a tube of melted and tempered bromcresol green dextrose agar.† These tubes were incubated at 37.5° C. and were read at 24, 48, and 72 hours. According to color change from blue-green to yellow, the tubes were graded from 0 to +4. Those tubes that showed no acid production as indicated by no color change in the medium were graded 0. Those tubes that showed high acid production as indicated by a complete change of color from blue-green to yellow were recorded as +4. The results of the 48-hour reading were used as the basis of the test, although the tubes were examined to see if any marked changes took place as early as 24 hours or as late as 72 hours.

This investigation was made possible through appropriations by the Oregon State Legislature in 1945 and 1947 for research under the direction of the Department of Foods and Nutrition, School of Home Economics. The Extension Women's Council of the State of Oregon was instrumental in obtaining the fund. The study was also a part of the Western Regional Research Project on the Nutritional Status of Population Groups in Selected Areas of Oregon with the cooperation of the Oregon Agricultural Experiment Station. The cooperation and assistance of the Bureau of Human Nutrition and Home Economics, U. S. Department of Agriculture, and the U. S. Public Health Service is acknowledged.

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*A preliminary study on Freshman students at Oregon State College in 1946-47[1] indicated that those who came from the Coast region showed the highest incidence of dental decay and those from the Central Oregon region showed the lowest incidence of dental decay. On the basis of this information, it was decided to study further in these two regions.

†Dried prepared agar was obtained from Difco Laboratories, Incorporated.

## RESULTS AND DISCUSSION

The means and ranges of the tests, divided according to counties are shown in Table I. Analyses of variance calculations* were made on this material to determine whether or not there were any significant county variations, ignoring age and sex differences. There were significant variations of pH, buffer capacity, ammonia nitrogen, and lactobacillus counts among counties. There were no significant variations of starch hydrolyzing time or Snyder test between counties.

TABLE I

MEANS AND RANGES OF TESTS ACCORDING TO COUNTIES

| REGION | COUNTY | MEAN AND RANGE | pH | STARCH HYDROLYZING TIME (SECONDS) | BUFFER CAPACITY | AMMONIA NITROGEN MG./100 ML. | LACTOBACILLUS COUNTS* | SNYDER TEST |
|---|---|---|---|---|---|---|---|---|
| Coast | Clatsop | Mean | 7.25 | 70 | 0.83 | 4.15 | 3.01 | 2.11 |
| | | Range | 6.60-7.70 | 15-900 | 0.1-2.7 | 0-9.99 | 0 - 7 | 0 - 4 |
| | Coos | Mean | 7.40 | 64 | 0.84 | 3.94 | 3.86 | 1.80 |
| | | Range | 6.55-7.93 | 15-600 | 0.1-3.6 | 0-9.99 | 0 - 7 | 0 - 4 |
| Central Oregon | Deschutes | Mean | 7.42 | 80 | 0.63 | 4.70 | 3.09 | 1.87 |
| | | Range | 6.68-7.97 | 30-900 | 0.1-1.6 | 0-9.99 | 0 - 7 | 0 - 4 |
| | Klamath | Mean | 7.37 | 66 | 0.70 | 6.02 | 2.94 | 1.82 |
| | | Range | 6.52-8.23 | 15-1350 | 0.2-1.7 | 0-9.99 | 0 - 7 | 0 - 4 |

*The lactobacillus counts were coded and grouped according to the following classification:

| | | |
|---|---|---|
| 0 | negative | per ml. of saliva |
| 1 | 1-999 | |
| 2 | 1,000-9,999 | |
| 3 | 10,000-99,999 | |
| 4 | 100,000-249,999 | |
| 5 | 250,000-499,999 | |
| 6 | 500,000-999,999 | |
| 7 | 1,000,000-1,499,999 | |
| 8 | 1,500,000-1,999,999 | |
| 9 | more than 2,000,000 | |

The means and ranges of the tests, further subdivided in regard to age and sex, are given in Table II. Analysis of variance calculations were made on these data in order to see if there were any significant differences in each county due to age or sex. These results were quite irregular, some being significant in some counties and not in others. In each county, however, there was a significant difference in buffer capacity between the sexes. In the case of the 14-year-olds in Clatsop County, the means are the same for the males and females. In every other instance the mean buffer capacity of the males is higher than that of the females. This was the most consistent significant variation in regard to either age or sex that was found.

Correlation coefficients were also calculated* between the various combinations of tests. These results are shown in Table III. There was a very highly significant correlation between lactobacillus counts and the Snyder test, i.e., high lactobacillus counts were found in combination with high acid production. There was also a highly significant correlation between pH and buffer capacity, indicating that high pH values were found in connection with higher buffer capacities. There were significant negative correlations between lactobacillus counts and ammonia nitrogen and between ammonia nitrogen and the Snyder test indicating inverse relationships between these pairs of analyses.

*The authors are indebted to Dr. Jerome C. R. Li, Associate Professor of Mathematics, for statistical analyses of the data.

TABLE II

THE MEANS AND RANGES OF THE VARIOUS CLINICAL TESTS CLASSIFIED ACCORDING TO COUNTY, SEX, AND AGE FOR 555 CHILDREN

| COUNTY | AGE | SEX | NO. CASES | pH | | STARCH HYDROLYZING TIME | | BUFFER CAPACITY | | AMMONIA NITROGEN | | LACTO-BACILLUS COUNT | | SNYDER TEST | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | MEAN | RANGE | MEAN (SECONDS) | RANGE (SECONDS) | MEAN | RANGE | MEAN (MG. %) | RANGE (MG. %) | MEAN | RANGE | MEAN | RANGE |
| **Coast Region** | | | | | | | | | | | | | | | |
| Clatsop | 14 | F | 12 | 7.25 | 6.70-7.50 | 91 | 15-600 | 0.78 | 0.2-1.8 | 4.05 | 0.00-7.56 | 3.17 | 0-7 | 2.42 | 0-4 |
| | 14 | M | 20 | 7.25 | 6.75-7.60 | 38 | 15-120 | 0.78 | 0.1-2.7 | 4.71 | 0.00-9.99 | 3.60 | 0-7 | 2.35 | 0-4 |
| | 15 | F | 16 | 7.20 | 6.60-7.70 | 40 | 15-150 | 0.66 | 0.1-1.4 | 3.48 | 0.00-9.24 | 3.06 | 0-7 | 2.31 | 0-4 |
| | 15 | M | 13 | 7.40 | 7.05-7.65 | 39 | 15- 90 | 1.23 | 0.4-2.3 | 4.04 | 0.00-9.99 | 2.46 | 0-7 | 1.92 | 0-4 |
| | 16 | F | 13 | 7.25 | 6.95-7.60 | 85 | 15-600 | 0.66 | 0.1-2.7 | 3.83 | 0.00-9.99 | 2.62 | 0-7 | 1.54 | 0-4 |
| | 16 | M | 18 | 7.14 | 6.75-7.55 | 126 | 15-900 | 0.78 | 0.3-2.3 | 4.52 | 0.00-8.96 | 3.06 | 0-7 | 2.11 | 0-4 |
| Coos | 14 | F | 34 | 7.41 | 6.98-7.75 | 68 | 30-300 | 0.71 | 0.1-1.2 | 3.06 | 0.00-9.99 | 3.79 | 1-7 | 2.21 | 0-4 |
| | 14 | M | 39 | 7.47 | 6.92-7.93 | 65 | 30-600 | 0.88 | 0.3-2.0 | 3.94 | 1.40-9.04 | 3.62 | 0-7 | 2.00 | 0-4 |
| | 15 | F | 30 | 7.28 | 6.85-7.86 | 70 | 30-600 | 0.58 | 0.1-1.6 | 4.09 | 0.00-7.42 | 4.37 | 2-7 | 1.43 | 0-4 |
| | 15 | M | 42 | 7.40 | 6.55-7.80 | 56 | 30-525 | 1.01 | 0.3-3.6 | 4.28 | 0.00-9.99 | 3.98 | 0-7 | 1.69 | 0-4 |
| | 16 | F | 26 | 7.42 | 6.77-7.77 | 48 | 15-150 | 0.77 | 0.1-2.0 | 4.10 | 0.00-9.99 | 3.35 | 2-6 | 1.12 | 0-4 |
| | 16 | M | 22 | 7.41 | 7.02-7.90 | 73 | 15-450 | 1.07 | 0.4-3.2 | 4.24 | 0.00-9.10 | 3.95 | 0-7 | 2.18 | 0-4 |
| **Central Oregon Region** | | | | | | | | | | | | | | | |
| Deschutes | 14 | F | 19 | 7.40 | 7.14-7.78 | 68 | 30-150 | 0.58 | 0.1-1.2 | 4.69 | 0.00-9.87 | 2.84 | 0-7 | 2.00 | 0-3 |
| | 14 | M | 10 | 7.56 | 7.12-7.79 | 59 | 30- 90 | 0.74 | 0.4-1.6 | 5.03 | 2.58-8.04 | 2.90 | 1-5 | 1.70 | 0-4 |
| | 15 | F | 17 | 7.48 | 6.97-7.74 | 112 | 30-900 | 0.51 | 0.1-1.2 | 4.65 | 0.00-9.99 | 3.06 | 0-7 | 1.53 | 0-4 |
| | 15 | M | 7 | 7.56 | 7.18-7.82 | 60 | 30-105 | 0.66 | 0.1-1.0 | 5.65 | 2.58-9.99 | 4.43 | 3-7 | 1.57 | 0-4 |
| | 16 | F | 21 | 7.29 | 6.79-7.65 | 97 | 30-750 | 0.53 | 0.1-1.6 | 4.13 | 0.00-7.81 | 3.00 | 0-7 | 2.00 | 0-4 |
| | 16 | M | 13 | 7.41 | 6.68-7.97 | 59 | 30- 90 | 0.95 | 0.3-1.5 | 4.89 | 2.32-9.87 | 2.92 | 0-5 | 2.23 | 0-4 |
| Klamath | 14 | F | 36 | 7.42 | 6.73-7.83 | 90 | 30-1350 | 0.57 | 0.2-1.1 | 5.90 | 1.96-9.99 | 2.75 | 0-7 | 1.64 | 0-4 |
| | 14 | M | 34 | 7.36 | 6.80-8.00 | 48 | 15-150 | 0.79 | 0.2-1.5 | 6.68 | 0.00-9.99 | 2.65 | 0-6 | 1.65 | 0-4 |
| | 15 | F | 30 | 7.34 | 6.52-7.75 | 62 | 30-150 | 0.57 | 0.2-1.6 | 5.56 | 0.00-9.99 | 2.53 | 0-6 | 1.70 | 0-4 |
| | 15 | M | 33 | 7.42 | 6.67-8.23 | 56 | 15-600 | 0.85 | 0.4-1.7 | 5.96 | 0.00-9.99 | 3.18 | 0-7 | 1.97 | 0-4 |
| | 16 | F | 25 | 7.22 | 6.57-7.67 | 85 | 30-600 | 0.60 | 0.2-1.6 | 6.03 | 0.00-9.99 | 2.64 | 0-6 | 1.84 | 0-4 |
| | 16 | M | 25 | 7.42 | 6.88-7.78 | 59 | 30-120 | 0.84 | 0.2-1.7 | 5.91 | 0.00-9.99 | 3.24 | 0-7 | 2.24 | 0-4 |

TABLE III

CORRELATION COEFFICIENTS FOR THE TESTS ON SALIVA*

| | STARCH HYDROLYZING TIME | BUFFER CAPACITY | AMMONIA NITROGEN | LACTO-BACILLUS COUNTS | SNYDER TEST |
|---|---|---|---|---|---|
| pH | +0.023 | +0.279† | +0.019 | -0.034 | -0.085 |
| Starch hydrolyzing time | | -0.067 | -0.041 | -0.006 | +0.014 |
| Buffer capacity | | | -0.018 | -0.056 | -0.037 |
| Ammonia nitrogen | | | | -0.130† | -0.107† |
| Lactobacillus counts | | | | | +0.349† |

*Significant values: 0.083 at the 5 per cent level
0.110 at the 1 per cent level

†Significant correlations.

The correlation between pH and buffer capacity was also highly significant on the results of salivary analyses conducted on a group of Freshman students at Oregon State College.[3] The correlation between lactobacillus counts and ammonia nitrogen in the study on freshman students, however, was not significant. Since the Snyder test was not performed on those students, no comparison can be made of the correlation coefficients involving this test.

## SUMMARY

Salivary analyses were made on 14-, 15- and 16-year-old native-born and native-reared children in two regions in Oregon. Examinations were made in Clatsop and Coos counties in the Coast Region and in Deschutes and Klamath counties in the Central Oregon Region.

The saliva samples were tested for pH, amylase activity as indicated by the starch hydrolyzing time, buffer capacity, ammonia nitrogen, lactobacillus counts, and acid producing power of the bacterial flora as indicated by the Snyder test.

Significant variations were found between counties in pH, buffer capacity, ammonia nitrogen, and lactobacillus counts. There was also a consistent significant variation in buffer capacity between the sexes.

Significant positive correlations were found between lactobacillus counts and the Snyder test and between pH and buffer capacity. Significant negative correlations were found between lactobacillus counts and ammonia nitrogen, and between ammonia nitrogen and the Snyder test.

Appreciation is expressed to the members of the Dental Advisory Committee, Dr. Harold J. Noyes, Dean, University of Oregon Dental School, Dr. O. T. Wherry, member of the Oregon State Board of Health, and Dr. E. R. Abbett, representative of the Oregon State Dental Association, for their helpful suggestions and criticisms in the planning of this study and the interpretation of the results. Appreciation is also expressed to Dr. Margaret L. Fincke, Head of the Department of Foods and Nutrition at Oregon State College, for numerous consultations.

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# EFFECT OF ZINC CHLORIDE AND POTASSIUM FERROCYANIDE ON PROTEOLYSIS

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ONE unsubstantiated theory[1] on the cause of dental caries is that bacteria enter "organic invasion roads" in the teeth, become established there, and destroy the tooth structure through proteolytic action. If this theory is correct, it is not unreasonable to assume that complete sealing of the ends of these "roads" would exclude bacteria and thus prevent tooth decay. To bring about such a sealing, impregnation of the tooth surface with zinc chloride and potassium ferrocyanide has been suggested.[2] Unfortunately, no laboratory evidence has been offered to indicate whether this impregnation procedure for preventing proteolytic destruction of the organic material of the tooth would have the effect which has been claimed for it. Therefore, it seemed worth while to test this point by studying the effect of zinc chloride and potassium ferrocyanide treatments on the breakdown of protein by trypsin. The use of trypsin seemed justified because similarity in the chemical processes occurring in the breakdown of proteins would make findings with this enzyme significant in respect to other enzyme systems.[3]

## EXPERIMENTAL

Three types of protein samples were used in an effort to test the effects of treatments with zinc chloride and potassium ferrocyanide on proteolysis. The laboratory equivalent of impregnation treatments[2] were made by immersing the samples in 40 per cent zinc chloride solution for one minute. This was followed by immersion and stirring in a solution of 20 per cent potassium ferrocyanide for one minute.* Following rinsing in tap water and distilled water, the specimens were incubated at 37° C. in a 2 per cent trypsin solution, buffered at pH 7.2 for twenty-four and forty-eight hours. A fresh trypsin solution was used for the second twenty-four hour period.

*Mett tubes.*—The first experiment was performed on egg albumin, coagulated and contained in open end Mett tubes, which were prepared by sucking raw albumin into capillary tubes and coagulating it by boiling in water. Since the measurement in this case was indicated by the number of millimeters of albumin that was liquefied, and since it was undesirable to have the albumin contaminated by the water in which it was heated, the tubes were scored with a file and broken after boiling, leaving uncontaminated albumin flush with the end of the tube.

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*The recommended clinical procedure[2] states that the potassium ferrocyanide should be applied until a precipitate forms, but since this always occurred immediately, acceptable laboratory standardization of method was obtained by allowing the solution to remain in contact with the samples for one minute.

These specimens were then treated with zinc chloride and potassium ferrocyanide by the method described above. In addition to trypsin, a series of tubes was run using a 2 per cent pepsin solution as the digesting enzyme. This enzyme was not used in later experiments because the enzyme solution had a pH of 4, which was outside the range of acidity likely to be found in the mouth and because later findings indicated that acid decalcification might cause discrepancies in findings made on tooth specimens.

After twenty-four hours' digestion, the Mett tubes showed that the albumin in the end of the tubes exposed to the enzymatic action had become clear. Measurement of the length of this clarified zone indicated the amount of digestion expressed in millimeters. Since the line of demarcation between digested and undigested albumin was not distinct, the accuracy of measurement was approximately one millimeter. Results after twenty-four hours showed an average of approximately one millimeter dissolution in the treated tubes for both the trypsin and pepsin solutions, with just under one millimeter in the control tubes. After forty-eight hours, the digestion averaged about 2 millimeters in the treated tubes, and about 1.5 millimeters in the control tubes (Table I).

TABLE I

DIGESTION OF EGG ALBUMIN IN METT TUBES

| | SAMPLE NO. | TRYPSIN | | PEPSIN | |
|---|---|---|---|---|---|
| | | 24 HRS. (MM.) | 48 HRS. (MM.) | 24 HRS. (MM.) | 48 HRS. (MM.) |
| Test | 1 | 1+ | 2- | 1 | 2+ |
| | 2 | 1 | 2 | 1- | 2 |
| | 3 | 1+ | 2+ | 1+ | 2+ |
| | 4 | 1- | 2+ | 1+ | 2- |
| | 5 | 1- | 2+ | 1+ | 2+ |
| | 6 | 1 | 2 | 1 | 2 |
| | 7 | 1+ | 2 | 1 | 2- |
| | 8 | 1+ | 2- | 1+ | 2+ |
| Control | 1 | 1 | 2 | 1+ | 2 |
| | 2 | 1- | 2- | 1- | 2- |
| | 3 | 1 | 2- | 1 | 2 |
| | 4 | 1+ | 2+ | 1- | 2 |

*Dentin.*—The second experiment was carried out on pieces of extracted teeth which were stored in a 50 per cent alcohol solution until used. These specimens were prepared from the root portions of extracted teeth which had been buffed with wet, coarse pumice to clean them and remove the periodontal membrane. After careful removal of the pulps with reamers, the roots were split into sections. So that the greater part of the surface would be dentin rather than cementum, the teeth were split transversely and, in a majority of instances, lengthwise as well. The specimens were then wiped dry and kept in a desiccator over water so that the moisture content would be as nearly constant as possible. Thereafter, they were weighed and placed in 95 per cent alcohol for two hours in order to remove excess moisture and to insure greater penetration of the experimental solutions. These samples were then treated with zinc chloride and potassium ferrocyanide and trypsin.

Results were calculated by figuring the percentage difference in the weight of the samples before and after chemical treatment and digestion. After the first twenty-four hours, there was a gain in weight of all specimens with an average of 1.20 per cent increase for the treated samples and 1.79 per cent increase for the control specimens. After forty-eight hours, the average over-all increase in weight was reduced to 0.42 per cent for the treated specimens and the average for the controls was 0.29 per cent (Table II).

TABLE II

EFFECT OF TRYPSIN DIGESTION ON WHOLE DENTIN

| SAMPLE NO. | | ORIGINAL WEIGHT (MG.) | AFTER 24 HR. WEIGHT (MG.) | DIGESTION % CHANGE | AFTER 48 HR. WEIGHT (MG.) | DIGESTION % CHANGE |
|---|---|---|---|---|---|---|
| Test | 1 | 290.6 | 292.0 | +0.48 | 291.5 | +0.31 |
| | 2 | 237.6 | 238.4 | +0.34 | 237.0 | -0.25 |
| | 3 | 198.6 | 199.3 | +0.35 | 198.6 | 0 |
| | 4 | 220.8 | 222.4 | +0.73 | 221.4 | +0.27 |
| | 5 | 144.5 | 147.8 | +2.28 | 146.0 | +1.04 |
| | 6 | 118.7 | 120.5 | +1.52 | 119.0 | +0.25 |
| | 7 | 128.9 | 130.5 | +1.24 | 129.0 | +0.08 |
| | 8 | 093.5 | 096.0 | +2.68 | 094.2 | +0.75 |
| Control | 1 | 210.0 | 210.6 | +0.29 | 210.0 | 0 |
| | 2 | 176.0 | 177.1 | +0.63 | 176.0 | 0 |
| | 3 | 119.0 | 120.5 | +1.26 | 119.8 | +0.67 |
| | 4 | 163.0 | 164.6 | +0.98 | 163.8 | +0.49 |

*Decalcified dentin.*—The third group was prepared exactly as the second, except that prior to weighing, the tooth sections were completely decalcified in a 1 normal hydrochloric solution. After the decalcification, the moisture content was kept as constant as possible so that it would not influence the weights. After weighing, both the control and test specimens were put in alcohol for dehydration, and following this zinc chloride and potassium ferrocyanide were applied to the test samples. So that the physical properties of the samples would influence the results as little as possible, particular care was taken to have the test and control specimens similar in weight and size.

TABLE III

EFFECT OF TRYPSIN DIGESTION ON DECALCIFIED DENTIN

| SAMPLE NO. | | ORIGINAL WEIGHT (MG.) | AFTER 24 HR. WEIGHT (MG.) | DIGESTION % CHANGE | AFTER 48 HR. WEIGHT (MG.) | DIGESTION % CHANGE |
|---|---|---|---|---|---|---|
| Test | 1 | 098.5 | 089.2 | - 9.5 | 075.0 | -23.9 |
| | 2 | 073.1 | 064.6 | -11.6 | 158.5 | -20.0 |
| | 3 | 058.2 | 053.8 | - 7.6 | 047.6 | -18.3 |
| | 4 | 065.4 | 062.4 | - 4.6 | 045.2 | -30.1 |
| | 5 | 056.6 | 052.4 | - 7.4 | 038.0 | -32.9 |
| | 6 | 069.0 | 068.1 | - 1.3 | 050.0 | -27.6 |
| | 7 | 065.0 | 060.7 | - 6.6 | 040.0 | -38.5 |
| | 8 | 062.0 | 052.0 | -16.2 | 037.5 | -39.6 |
| Control | 1 | 061.9 | 056.3 | - 9.1 | 048.2 | -21.2 |
| | 2 | 072.3 | 063.2 | -12.6 | 056.3 | -22.1 |
| | 3 | 060.0 | 053.9 | -10.2 | 047.5 | -20.8 |
| | 4 | 066.2 | 060.8 | - 8.2 | 048.0 | -27.4 |

The results for the decalcified dentin specimens were calculated in the same manner as for the undecalcified dentin specimens. These teeth, however, showed a loss in weight, with the treated teeth losing an average of 8.1 per cent and the control teeth averaging 10.0 per cent loss. After forty-eight hours, the average loss was 28.8 per cent for the treated teeth and 22.9 per cent for the controls. Because of the spongy nature of this test material, it is felt that loss of water resulting from unavoidable pressures exerted on the specimens may have influenced unfavorably the accuracy of the results.

## DISCUSSION

It will be noted from the experiment with the Mett tubes, that the protein samples treated with zinc chloride and potassium ferrocyanide showed more digestion than the untreated ones. Since enzymatic digestion of proteins is brought about by the breakdown of protein molecules into component amino acids, a possible explanation for our findings may be that zinc chloride of itself causes breakdown of the protein. That it does have such a denaturing effect is suggested by the observation that during treatment the albumin in the tubes became whiter and more opaque when placed in the zinc chloride solution. Thus, if the molecular breakdown was already started by the action of the zinc chloride, it is understandable that it may have enhanced the effect of the proteolytic enzymes.

The results obtained with the whole dentin specimens deserve comment. In view of differences in the size and surface of the specimens, the variations in the weight changes are not surprising and are within reasonable limits of experimental error. An unexpected finding is that both the control and test samples showed an over-all increase in weight. This presumably is the result either of increase in their moisture content or of an adsorption of trypsin on their surfaces. The fact that the treated samples showed a greater weight increase than the controls is possibly the result of the formation of a precipiate during chemical treatment.

The above findings indicate that very little, if any, proteolysis took place in the whole dentin. This is in direct contrast to what happened when the decalcified dentin was subjected to the action of trypsin, in which case there was a considerable weight loss. The explanation for this contrasting effect would seem to be that removal of the calcium salts is necessary before proteolysis can occur. This conclusion is essentially that offered by Goadby[4] almost fifty years ago. It is a finding which has also been confirmed by Burnett[5] using proteolytic bacteria isolated from the mouth.

The failure to demonstrate any inhibiting effect on proteolysis as the result of treatments with zinc chloride and potassium ferrocyanide and even the evidence of an enhancement of protein destruction by such treatments is not surprising and is in keeping with theoretically predictable results. This conclusion naturally raises doubts as to whether these agents can bring about the effect on tooth protein which has been claimed for them. While it is the author's opinion that they almost certainly do not have such an effect, our findings do not con-

vince us that careful treatments of the tooth surface with zinc chloride and potassium ferrocyanide (or a variety of other chemicals) might not reduce the activity of dental caries. We do feel, however, that if any caries reduction is brought about by these agents, it is probably the result of factors other than interference with proteolysis of dental tissues.[6]

## SUMMARY

Zinc chloride and potassium ferrocyanide were tested on laboratory specimens of egg albumin and dentin to determine their effects on trypsin proteolysis. It was found that proteolysis of egg albumin was enhanced by these agents. Normal dentin was not affected by trypsin. Decalcified dentin was digested to the same extent with or without treatment with zinc chloride and potassium ferrocyanide.

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# EFFECT OF FLUORIDES AND OTHER COMPOUNDS ON THE SOLUBILITY OF ENAMEL, DENTIN, AND TRICALCIUM PHOSPHATE IN DILUTE ACIDS

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IT was in 1942 that Cheyne[1] using potassium fluoride and Bibby[2] using the sodium salt first showed that fluoride applied topically to the teeth would reduce tooth decay. The efforts of Galagan and Knutson[3] and others of the United States Public Health Service, using sodium fluoride, verified these findings. In 1947 the Council on Dental Therapeutics of the American Dental Association[4] gave wholehearted approval to the use of topical sodium fluoride as a therapeutic measure in the control of caries by the dentist.

As a part of the development of these procedures, Volker[5] in 1939 described a gravimetric method for measuring enamel and dentin solubility. He was able to show that when these tissues were treated with sodium fluoride they became less soluble. In 1945 Buonocore and Bibby[6] showed that lead fluoride was more effective than sodium fluoride in reducing the solubility of enamel, in vitro. It is obvious, however, that the method they developed is inadequate for the measurement of this effect when lead fluoride is used.* Consequently, when Galagan and Knutson[3] attempted to use lead fluoride clinically to reduce tooth decay, it was found less effective than sodium fluoride. Because of the difficulties associated with the method described by Buonocore and Bibby[6] modifications were made by Muhler and Van Huysen.[7] These methods with their modifications have been essentially that of a comparison of weight losses[6, 7] that occurred when unprotected and protected powdered enamel are combined with an acetate buffer.

This paper describes a study of a new and more precise method for determining changes in the solubility of enamel, dentin, and tricalcium phosphate in an acetate buffer. The solubility of the unprotected and protected (reagent-treated) tooth tissues and tricalcium phosphate is determined by measuring the amount of calcium and phosphorus dissolved in unit time when the tissues are dispersed in weak acid of constant strength. The new method is used because, although there is a general tendency to call loss in weight decalcification, it was felt that a volumetric determination of calcium and phosphorus in the filtrates is a more specific measure of this phenomenon. Rae and Clegg[8, 9] have investigated in a similar manner the effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel, and whole teeth in unbuffered 1 per cent lactic acid.

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*The lead ion added to the tooth tissue during the protection stage of the process produces in itself an excess of weight which is not compensated for in the decalcification phase of the experiment.

## PROCEDURE

The description of the procedures used in this study is divided into three sections. This division is a convenience used only for the purpose of describing more clearly the various parts of the method. The three sections are: (1) decalcification of unprotected powdered enamel; (2) reagent treatment (protection) and decalcification of powdered enamel; and (3) calcium and phosphorus analysis of the filtrates from Sections 1 and 2.

*Section 1. Decalcification of Unprotected Powdered Enamel.*

The first step in the process of studying the effects of reagents upon solubility of a substance consists naturally of determining the solubility of untreated material. Briefly this is brought about by mixing powdered enamel, dentin, and tricalcium phosphate for 40 minutes with acetic acid at pH 4.00 and analyzing the filtrates for Ca and P. Since the procedures applied to the examination of the solubility of enamel, dentin, and tricalcium phosphate are the same, only that part of the experiment in which the enamel is used is described.

Into a 50-ml. centrifuge tube is placed 0.1000 Gm. from a large stock of powdered enamel prepared by the flotation method as outlined by Manly and Hodge.[10] The particle size of the enamel and dentin used is such that it passed a 100- and remained on a 140-mesh screen after long vibration on a Kerr vibrator. There is then added to the centrifuge tube 25 ml. of 0.2N acetate buffer* and the contents are shaken in an automatic shaker for 40 minutes. The automatic shaker is designed so that it thoroughly mixes the solid with the liquid phase. The tube and contents are then centrifuged at 1500 r.p.m. for 3 minutes and the liquid phase decanted through a Selas porcelain filter.† The enamel is washed twice with 3 ml. of fluorine-free distilled water‡ and centrifuged and decanted through the filter after each washing. The washings are added to the first filtrate. The combined filtrate is made to a volume of 50 ml. with glass-distilled water. This filtrate from unprotected enamel decalcified with acetate buffer for 40 minutes is immediately analyzed for Ca and P according to the method outlined in Section 3.

*Section 2. Reagent Treatment (Protection) and Decalcification of Powdered Enamel.*

This second section of the study is concerned with two overlapping phases. There is, first, the procedure used for the reagent treatment (protection) of the tissue. This is accomplished by stirring the enamel for 20 minutes with the reagent, buffered to pH of 4.00,[11, 12] and, second, 20 additional minutes of decalcification with acetic acid. Since the reagent used to treat or protect the

*The 0.2N acetate buffer, pH 4.00, was prepared from sodium hydroxide, glacial acetic acid, and fluorine-free water.

†A unique problem existed pertaining to the type of filtering crucible originally used in the gravimetric analysis. The crucible should have been porous enough to permit the liquid to be removed by filtration but still dense enough to hold the liquid while being stirred. This objective was not fulfilled, so a slight error was probably incorporated in the older method. In the gravimetric technic, the Selas crucible was placed in a plain porcelain crucible and the liquid which filtered through in 20 minutes was transferred to the volumetric flask with the filtrate. The amount of liquid that filtered through the Selas crucible into the porcelain one varied with the crucible being used and was approximately from one drop to 3 milliliter. Obviously, in the volumetric technic there is no concern for leakage through the crucibles. The subject is mentioned only to illustrate a source of error in the gravimetric analysis not found in this method.

‡Fluorine-free water was prepared by redistilling distilled water in an alkaline permanganate solution. Actually, this water may not be fluorine free, so it should be referred to as glass-distilled water.

enamel is adjusted to a pH of 4.00, decalcification also takes place during the protection phase. The reagent-treated tissues therefore undergo the same 40-minute decalcification period as the unprotected enamel.

A. *Protection of enamel with buffered reagent for 20 minutes.*—Into a 50-ml. centrifuge tube is placed 0.1000 Gm. of powdered enamel from the stock supply. The 0.1000 Gm. sample of enamel is combined with 5 ml. of one of the reagent solutions. The centrifuge tube and contents are then shaken for 20 minutes at room temperature. Any debris remaining on the sides of the tube is washed with glass-distilled water and the tube and contents centrifuged at 1500 r.p.m. for 3 minutes. The clear fluid is removed by decantation and filtered through a Selas crucible. The filtrate is transferred to a 50-ml. volumetric flask. The centrifuge tube and contents are washed twice with glass-distilled water. This is called the reagent filtrate. Since decalcification is brought about during this 20-minute application of the acidulated protecting filtrates, this fluid is analyzed for Ca and P simultaneously with the decalcification filtrate from Part B of the procedure given below. The unprotected enamel solubility is determined by 40-minute decalcification so that it is necessary for comparison of the solubility of this protected enamel to decalcify it for an additional 20 minutes in acetic acid. This provides a total of 40 minutes' decalcification for both unprotected and protected enamel.

B. *Decalcification of the reagent-treated enamel for an additional 20 minutes.*—After removal of the liquid phase (reagent filtrate) from the protected enamel sample, 20 ml. of 0.2N acetate decalcifying solution are placed in the centrifuge tube. This is shaken for 20 minutes and the liquid phase is removed as previously described. The filtrate from this part of the procedure is the decalcification filtrate and is also made to a volume of 50 ml. with glass-distilled water. Samples from A (reagent filtrate) and B (decalcification filtrate) are then analyzed for Ca and P simultaneously according to the procedure outlined below in Section 3.

*Section 3. Calcium and Phosphorus Analysis of Filtrates from Sections 1 and 2.*

Three filtrates are obtained from Sections 1 and 2 of the foregoing procedures. The first is a total decalcification filtrate, obtained from the solution of 0.1000 Gm. of unprotected enamel shaken for 40 minutes with the 0.2N acetate buffer. The second and third filtrates are: (A) The reagent decalcification filtrate that was obtained from 20 minutes of treatment of the powdered enamel with an acidulated protecting reagent solution; and (B) the decalcification filtrate that was obtained following another 20 minutes of decalcification with acetate buffer of the enamel protected in A.

The following descriptions are of the calcium and phosphorus analytical methods applied to these three filtrates. The description applies only to enamel but it must be remembered that the same was applied to filtrates from dentin and tricalcium phosphate.

### CALCIUM DETERMINATION

The calcium is precipitated directly from the filtrate as oxalate and the oxalate is titrated with potassium permanganate. Sendroy[13] has shown that

in similar calcium analysis with blood it is not necessary to remove the protein. In addition, because the protein content of enamel is small, no attempt was made here to remove this component. The calcium method described in detail below is used even though it is felt that it has definite weaknesses. Certain ions used in the reagent protection of enamel and dentin may interfere in the determination of calcium as the oxalate, unless all the excess of such ions is removed. These include Mg, Ba, Sr, Mn, and Al. The method, with certain modifications, is that of Clark and Collip.[14, 17] The details for procedure are as follows:

Introduce into a graduated 15-ml. Pyrex centrifuge tube 2 ml. of the enamel filtrate, 2 ml. of glass-distilled water, 1 ml. of 4 per cent ammonium oxalate solution and 0.2 ml. of Triton.*[15] The pH is adjusted to the end point of bromcresol green. The contents are then mixed thoroughly by tapping the outside of the tube. The tube and contents are allowed to stand for one-half hour or longer at room temperature. Again the contents of the tube are mixed by tapping. They are then centrifuged for 5 minutes at 1,500 revolutions per minute. The liquid phase is removed by suction.

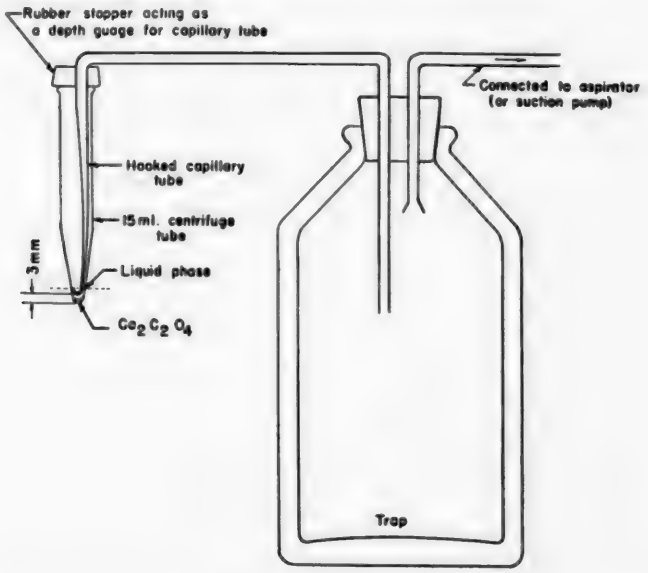


Fig. 1.—Capillary suction apparatus.

It was found during the early part of the work that suction was a more satisfactory method for removing the liquid than decantation. Therefore, a piece of glass tubing was bent in such a form that the curved nozzle end was at right angles to the stem attached to the suction tube. The glass tubing was shaped so that it would remove all but 0.2 ml. of the liquid over the calcium oxalate and still not disturb the oxalate pad. A rubber stopper was used to make certain that the tube did not touch the oxalate (Fig. 1).

*Triton, N. E.: 1:30 dilution of the original solution obtained from Rohm and Haas Co., Washington Square, Philadelphia, Pa.

The precipitate and sides of the tube are washed with 3 ml. of dilute ammonia (2 per cent) and centrifuged as before. After removal of the liquid by suction a second 3 ml. of dilute ammonia, plus 0.2 ml. of Triton are added. The contents are mixed and centrifuged. The liquid phase is then removed in the usual manner. This precipitate has added to it 2 ml. of 1N sulfuric acid, introduced by blowing it from a pipette directly upon the precipitate. The tube and contents are placed in a hot water bath for one minute and titrated with 0.01 N $KMnO_4$ to a definite pink color that persists for at least one minute. The temperature of the sample is kept at 70° C. to 75° C. during the titration.

### PHOSPHORUS DETERMINATION

The method for phosphorus is that of Fiske and Subbarow:[16, 17] Pipette 2 ml. of enamel filtrate taken as a representative sample from the 50-ml. volumetric flask into a 25-ml. volumetric flask. Add 2.5 ml. of molybdate reagent and enough glass-distilled water to make a volume of 10 ml. Add 1 ml. of 1, 2, 4-aminonaphthosulfonic acid reagent and make to a volume of 25 ml. with glass-distilled water. At the end of 5 minutes compare in a photoelectric colorimeter with a standard simultaneously prepared.

### DATA

This study is designed to show the comparative effect of 32 reagents upon the solubility of powdered enamel, dentin, and tricalcium phosphate. Although an attempt was made to make solutions of these reagents as uniform as possible, slight differences do occur. Table I shows in the second column the grams of solute placed in each 500 ml. of solvent. The third column shows the concentration, in normality, of the reagent solutions used. The fourth column gives the molecular weights of the reagents and the fifth, the pH of the solutions after adjustment. All the reagents used in this study are made to a 1/20 normality except lead fluoride, which due to its insolubility is 1/200 normal. The pH of the 0.2N acetate buffer is 4.0 as are the values of all other solutions with the exception of stannous chloride which is 3.10. In attempting to adjust this reagent to pH 4.00, so much (about 20 ml.) of the 1N NaOH is required that the precipitated stannous hydroxide makes the preparation unusable. The pH, however, may be changed slightly from 2.6 to 3.10 by the addition of 1N NaOH. It is difficult, due to the amphoteric properties of the solution, to predict how much 1N NaOH must be added to the stannous chloride solution to produce a pH of 3.10. Ten milliliters of the 1N NaOH added to 500 ml. of the stannous chloride is enough to produce a flocculent precipitate. When this is permitted to stand for about 2 hours a clear fluid is obtained with a pH of 3.10. Consideration was given to this difference of pH when evaluating the data.

*Calcium and phosphorus.*—The average values for the Ca and P removed from the unprotected enamel, dentin, and tricalcium phosphate are found in the first or incomplete rows of Tables II, III, and IV in columns 6 and 7, under the heading Decalcification Total. These first row values are the amounts of Ca and P found in the filtrates when the unprotected materials were combined with 0.2N $CH_3COOH$ at a pH of 4.00 for a period of 40 minutes. In each instance the values represent an average of 12 determinations. The powdered

enamel lost 12.70 ± 0.1 mg. of Ca and 6.00 ± 0.1 mg. of P (Table II); the powdered dentin lost 14.75 ± 0.1 mg. Ca and 6.80 ± 0.1 mg. of P (Table III); and the tricalcium phosphate lost 20.13 ± 0.1 mg. of Ca and 9.99 ± 0.1 mg. of P (Table IV) to each of the respective filtrates. The average values for Ca and P lost from the respective unprotected samples of enamel, dentin, and tricalcium phosphate are placed at the head of rows 6 and 7 of each table so that one can determine the solubility reducing effect brought about by the respective reagents by comparing the remainder of the values directly with those in the first row.

TABLE I

THE VARIOUS REAGENTS USED, THEIR CONCENTRATION AND MOLECULAR WEIGHTS

| REAGENT SOLUTION | GM./500 ML. | NORMALITY | MOLECULAR WEIGHT | ADJUSTED pH |
|---|---|---|---|---|
| $CH_3COOH$ | 6.30 | 1/5 | 60.00 | 4.00 |
| $NaF$ | 1.05 | 1/20 | 42.00 | 4.00 |
| $AgNO_3$ | 4.25 | 1/20 | 153.89 | 4.00 |
| $PbF_2$ | 0.32 | 1/200 | 245.21 | 4.00 |
| $Pb(NO_3)_2$ | 4.14 | 1/20 | 331.23 | 4.00 |
| $SnF_2$(III) | 1.96 | 1/20 | 156.70 | 4.00 |
| $SnF_2$(II) | 1.96 | 1/20 | 156.70 | 4.00 |
| $SnF_2$(I) | 1.96 | 1/20 | 156.70 | 4.00 |
| $CH_2ICOOH$ | 4.65 | 1/20 | 185.92 | 4.00 |
| $NaI$ | 3.75 | 1/20 | 149.92 | 4.00 |
| $ZnF_2$ | 1.29 | 1/20 | 103.38 | 4.00 |
| $UO_2(NO_3)_2.6H_2O$ | 6.28 | 1/20 | 502.18 | 4.00 |
| $CuF_2$ | 1.27 | 1/20 | 101.57 | 4.00 |
| $KIO_3$ | 5.35 | 1/20 | 214.04 | 4.00 |
| $CrF_2$ | 2.25 | 1/20 | 90.01 | 4.00 |
| $BF_3$ | 0.57 | 1/20 | 67.82 | 4.00 |
| $NH_4HF_2$ | 1.42 | 1/20 | 57.05 | 4.00 |
| $NH_4I$ | 3.62 | 1/20 | 144.96 | 4.00 |
| $UO_2(C_2H_3O_2).2H_2O$ | 5.30 | 1/20 | 424.19 | 4.00 |
| $Na_2C_2O_4$ | 1.68 | 1/20 | 134.01 | 4.00 |
| $(NH_4)_2C_2O_4.H_2O$ | 2.79 | 1/20 | 142.12 | 4.00 |
| $K_2C_2O_4.H_2O$ | 2.30 | 1/20 | 184.21 | 4.00 |
| $(COOH)_2.2H_2O$ | 1.57 | 1/20 | 126.07 | 4.00 |
| $SnCl_2$ | 2.82 | 1/20 | 225.65 | 3.10 |
| $KF$ | 2.35 | 1/20 | 58.10 | 4.00 |
| $LiF$ | 0.67 | 1/20 | 25.94 | 4.00 |
| $NH_4F$ | 0.93 | 1/20 | 37.04 | 4.00 |
| $SrF_2$* | 1.57 | 1/20 | 125.60 | 4.00 |
| $MgF_2$* | 0.78 | 1/20 | 62.32 | 4.00 |
| $CuF_2$* | 1.27 | 1/20 | 101.57 | 4.00 |
| $CdF_2$* | 1.88 | 1/20 | 150.00 | 4.00 |
| $NaHF_2$* | 0.78 | 1/20 | 62.00 | 4.00 |
| $SrF_2$ | 15.70 | 1/20 | 125.63 | 4.00 |

III Secured from A. D. McKay, New York, N. Y.
II Secured from General Chemical Company, New York, N. Y.
I Secured from Harshaw Chemical Company, Cleveland, Ohio
*Secured from Harshaw Chemical Company, Cleveland, Ohio

Tables II, III, and IV show in the first column the reagents whose chemical characteristics are given in Table I. Columns 2 and 3 of Tables II, III, and IV are grouped into a section labeled Reagent Filtrate. Column 2 gives the milligrams of Ca and column 3 the milligrams of P found in the filtrates obtained when each acidulated reagent solution was mixed for 20 minutes with the samples of enamel, dentin, and tricalcium phosphate.

The columns 4 and 5 of Tables II, III, and IV are grouped together in a section labeled Decalcification Filtrate which shows the data obtained when the

TABLE II

CALCIUM AND PHOSPHORUS IN ENAMEL FILTRATE EXPRESSED IN MG. OF CA AND P DISSOLVED FROM SAMPLE

| REAGENT SOLUTION | REAGENT FILTRATE 20 MINUTES | | DECALCIFICATION FILTRATE 20 MINUTES | | DECALCIFICATION TOTAL 40 MINUTES | |
|---|---|---|---|---|---|---|
| | CALCIUM | PHOSPHORUS | CALCIUM | PHOSPHORUS | CALCIUM | PHOSPHORUS |
| None | (Filtrate from unprotected enamel contained) | | | | 12.70 | 6.00 |
| NaF | 2.05 | 2.25 | 5.16 | 2.03 | 8.31 | 4.28 |
| $AgNO_3$ | N.S.R. | N.S.R.† | 8.90 | 3.90 | 8.90 | 3.90 |
| $PbF_2$ | 1.75 | 0.90 | 3.07 | 1.54 | 4.82 | 2.44 |
| $Pb(NO_3)_2$ | 1.50 | 0.07 | 2.40 | 2.23 | 3.90 | 2.40 |
| $SnF_2$(III) | 1.99 | 0.98 | 2.71 | 1.52 | 4.70 | 2.50 |
| $SnF_2$(II) | 0.60 | 0.81 | 1.25 | 1.09 | 3.55 | 1.90 |
| $SnF_2$(I) | 1.50 | 0.90 | 1.75 | 0.90 | 3.25 | 1.80 |
| $CH_2ICOOH$ | 1.83 | 1.17 | 5.72 | 3.00 | 7.55 | 4.17 |
| NaI | 1.95 | 0.15 | 6.45 | 2.79 | 8.40 | 2.94 |
| $ZnF_2$ | 0.35 | 0.43 | 4.78 | 2.35 | 5.15 | 2.78 |
| $UO_2(NO_3)_2.6H_2O$ | 0.15 | N.S.R. | 5.07 | 2.98 | 5.22 | 2.98 |
| $CuF_2$ | N.S.R. | 0.74 | 4.15 | 2.66 | 4.15 | 3.40 |
| $KIO_3$ | 0.54 | 1.35 | 6.06 | 2.13 | 6.60 | 3.48 |
| $CrF_2$ | N.S.R. | 0.27 | 5.10 | 2.07 | 5.10 | 2.34 |
| $BF_3$ | 0.45 | 0.78 | 5.07 | 2.66 | 5.52 | 3.44 |
| $NH_4HF_2$ | 1.50 | 2.53 | 5.84 | 1.45 | 6.34 | 3.98 |
| $NH_4I$ | 1.70 | 0.66 | 10.35 | 5.79 | 11.05 | 6.45 |
| $UO_2(AC).2H_2O$ | N.S.R. | N.S.R. | 5.98 | 2.47 | 5.98 | 2.47 |
| $Na_2C_2O_4$ | 0.15* | 1.06 | 5.08 | 2.03 | 5.23 | 3.09 |
| $(NH_4)_2C_2O_4.H_2O$ | 0.85* | 1.90 | 8.40 | 5.00 | 9.25 | 6.90 |
| $K_2C_2O_4.H_2O$ | N.S.R.† | 2.11 | 8.80 | 3.87 | 8.80 | 5.98 |
| $(COOH)_2.2H_2O$ | 1.40* | 3.10 | 9.00 | 4.00 | 10.40 | 7.10 |
| $SnCl_2$ | 1.55 | 0.87 | 3.00 | 1.23 | 4.45 | 2.10 |
| KF | 0.50 | 0.82 | 3.50 | 2.98 | 4.00 | 3.80 |
| LiF | 2.40 | 1.25 | 6.18 | 1.47 | 6.58 | 2.02 |
| $NH_4F$ | N.S.R. | 0.95 | 5.50 | 1.81 | 5.50 | 2.86 |
| $MgF_2$(H) | 3.35* | 1.02 | 10.00 | 4.10 | 13.35 | 5.12 |
| $CuF_2$(H) | N.S.R. | N.S.R. | 4.00 | 3.35 | 4.00 | 3.35 |
| $CdF_2$(H) | 0.65 | N.S.R. | 5.49 | 1.90 | 6.14 | 1.90 |
| $NaHF_2$(H) | 1.20* | 0.92 | 5.10 | 1.78 | 6.30 | 2.70 |
| KF(H) | 0.90 | 0.68 | 5.10 | 1.24 | 6.20 | 5.92 |
| $SrF_2$(H) | 0.85* | N.S.R. | 4.95 | 2.96 | 5.80 | 2.96 |

N.S.R. Not sufficient recovery.
*Interference was noted. Calculated directly from P data.
†Interference was noted. Calculated directly from Ca/P ratio.

reagent-treated enamel, dentin, and tricalcium phosphate are combined with 0.2N acetate buffer at pH 4.00 for an additional 20 minutes.

Table V* is a comparison of the six most satisfactory reagents used to protect enamel, dentin, and tricalcium phosphate against decalcification. Table II shows for instance that stannous fluoride† of all the reagents studied has the greatest effect in reducing the solubility of enamel. A summary of this data arranged in Table V shows in the first row of values of column 1 that 3.25 mg. calcium and 1.80 mg. phosphorus were removed from 0.1000 Gm. of protected enamel. This is compared with the values shown in the bottom row of column 1, Table V, where one can see that 12.70 mg. of Ca and 6.00 mg. P are found in the filtrate when enamel is not protected by a reagent. Keeping in mind

*The data in Table V could be arranged in various ways. For instance one could list the reagent compounds according to their effectiveness in controlling either the solubility of Ca or P. It was considered more convenient in this study to arrange this table to show the order of their effectiveness in reducing the solubility of enamel, dentin, and $Ca_3(PO_4)_2$.

†Secured from the Harshaw Chemical Company, Cleveland, Ohio.

TABLE III

CALCIUM AND PHOSPHORUS IN DENTIN FILTRATE EXPRESSED IN MG. OF CA AND P DISSOLVED FROM SAMPLE

| PROTECTING REAGENTS | REAGENT FILTRATE 20 MINUTES | | DECALCIFICATION FILTRATE 20 MINUTES | | DECALCIFICATION TOTAL 40 MINUTES | |
|---|---|---|---|---|---|---|
| | CALCIUM | PHOSPHORUS | CALCIUM | PHOSPHORUS | CALCIUM | PHOSPHORUS |
| None | (Filtrate from unprotected dentin contained) | | | | 14.75 | 6.80 |
| NaF | 2.80 | 2.61 | 5.84 | 2.67 | 8.64 | 5.28 |
| $AgNO_3$ | 4.95 | 3.40† | 9.05 | 2.63 | 14.00 | 5.03 |
| $PbF_2$ | 3.40 | 0.48 | 3.40 | 0.72 | 6.80 | 1.20 |
| $Pb(NO_3)_2$ | 1.95 | 0.11 | 3.65 | 1.78 | 5.60 | 1.89 |
| $SnF_2$(III) | 0.91 | 0.40 | 3.65 | 2.05 | 4.56 | 2.45 |
| $SnF_2$(II) | 0.45 | N.S.R. | 4.25 | 2.03 | 4.70 | 2.03 |
| $SnF_2$(I) | 0.15 | N.S.R. | 4.50 | 1.90 | 4.65 | 1.90 |
| $CH_3ICOOH$ | 2.10 | 1.81 | 9.32 | 4.07 | 11.42 | 5.88 |
| NaI | 0.45 | 0.76 | 13.50 | 5.09 | 13.95 | 5.85 |
| $ZnF_2$ | 3.50 | 0.90 | 5.20 | 2.85 | 8.70 | 3.75 |
| $UO_2(NO_3)_2.6H_2O$ | 2.05 | N.S.R. | 11.45 | 4.94 | 13.50 | 4.94 |
| $CuF_2$ | N.S.R. | 1.20 | 6.60 | 1.20 | 6.60 | 2.40 |
| $KIO_3$ | 0.70 | 0.54 | 7.05 | 3.81 | 7.75 | 4.35 |
| $CrF_2$ | 0.65 | 0.47 | 7.85 | 3.33 | 8.50 | 3.80 |
| $BF_3$ | N.S.R. | 0.74 | 12.00 | 3.42 | 12.00 | 4.16 |
| $NH_4HF_2$ | 0.35 | 1.78 | 2.95 | 4.63 | 6.20 | 6.41 |
| $NH_4I$ | 0.25 | 0.58 | 11.75 | 5.93 | 12.00 | 6.51 |
| $UO_2(Ac).2H_2O$ | 1.25 | N.S.R. | 13.95 | 4.03 | 15.20 | 4.03 |
| $Na_2C_2O_4$ | 0.35* | 1.74 | 7.15 | 2.18 | 7.50 | 3.92 |
| $(NH_4)_2C_2O_4$ | 0.25* | 2.71 | 0.10 | 4.49 | 9.35 | 7.20 |
| $K_2C_2O_4.H_2O$ | 1.35* | 1.74 | 10.40 | 4.51 | 11.75 | 6.25 |
| $(COOH)_2.2H_2O$ | 0.45* | 1.78 | 6.65 | 2.61 | 7.10 | 4.40 |
| $MgF_2$(H) | 2.45† | 0.88 | 6.85 | 2.62 | 9.30 | 3.50 |
| $SnCl_2$ | 0.35 | 0.06 | 4.73 | 2.62 | 5.08 | 2.68 |
| KF | N.S.R. | 0.66 | 5.40 | 1.89 | 5.40 | 2.55 |
| LiF | N.S.R. | 1.24 | 6.00 | 2.82 | 6.00 | 4.06 |
| $NH_4F$ | 0.95 | 0.98 | 6.45 | 3.35 | 7.40 | 4.33 |
| $NaHF_2$(H) | 0.95† | 0.76 | 7.35 | 2.96 | 8.30 | 3.62 |
| KF(H) | 0.15 | 0.29 | 7.45 | 2.46 | 7.60 | 2.75 |
| $CdF_2$(H) | 1.35 | N.S.R. | 9.35 | 3.81 | 10.70 | 3.81 |
| $CuF_2$(H) | 0.25 | N.S.R. | 5.45 | 2.65 | 5.70 | 2.65 |
| $SrF_2$(H) | 0.30† | N.S.R. | 10.30 | 4.50 | 10.60 | 4.80 |

N.S.R. Not sufficient recovery.
*Interference was noted. Calculated directly from P data.
†Interference was noted. Calculated directly from Ca/P ratio.

again the amount of inorganic substance (Ca + P = 18.70 mg.) removed from unprotected enamel, it is of interest to compare this with the protection afforded by the next compound listed in Table V or stannous fluoride II.* The soluble part of enamel protected by this fluoride is 3.55 mg. Ca and 1.90 mg. of P. Further examination of Table V shows that when enamel is protected with stannous chloride there is a total of 6.55 mg. lost to the filtrate; for lead nitrate, the soluble amounts of Ca and P is 6.30 mg.; for stannous fluoride III,† it is 7.20 mg.; and for sodium fluoride, 12.59 mg.

Again the data in Table V show that stannous fluoride I is the most effective protective reagent in respect to dentin solubility. There is a total loss to the filtrate of 6.55 mg. of Ca and P. Stannous fluoride II follows this for there is a total loss of 6.73 mg. Stannous fluoride III is the third most effective reagent with a total loss of 7.01 mg. Lead nitrate is the fourth most effective

*Secured from Baker & Adamson, Division of General Chemical Co., New York, N. Y.
†Secured from A. D. McKay, New York, N. Y.

TABLE IV

CALCIUM AND PHOSPHORUS IN TRICALCIUM PHOSPHATE FILTRATES EXPRESSED IN MG. OF CA AND P DISSOLVED FROM SAMPLES

| REAGENT SOLUTION | REAGENT FILTRATE 20 MINUTES | | DECALCIFICATION FILTRATE 20 MINUTES | | DECALCIFICATION TOTAL 40 MINUTES | |
|---|---|---|---|---|---|---|
| | CALCIUM | PHOSPHORUS | CALCIUM | PHOSPHORUS | CALCIUM | PHOSPHORUS |
| None | (Filtrate from unprotected tricalcium phosphate contained) | | | | 20.13 | 9.99 |
| NaF | 7.05 | N.S.R. | 7.95 | 3.24 | 15.00 | 3.24 |
| $AgNO_3$ | 0.33 | † | 16.50 | † | 16.88 | |
| $PbF_2$ | 3.85 | N.S.R. | 11.15 | 6.97 | 15.00 | 6.97 |
| $Pb(NO_3)_2$ | 4.00 | N.S.R. | 11.00 | 3.17 | 15.00 | 3.17 |
| $SnF_2$(III) | 2.05 | N.S.R. | 7.95 | 5.70 | 7.75 | 5.70 |
| $SnF_2$(II) | 1.25 | N.S.R. | 6.75 | 3.03 | 8.00 | 3.03 |
| $SnF_2$(I) | 0.50 | N.S.R. | 4.60 | 0.20 | 2.10 | 0.20 |
| $CH_2ICOOH$ | 3.23 | 0.76 | 13.45 | 8.61 | 16.68 | 9.37 |
| NaI | 1.60 | N.S.R. | 14.40 | 7.02 | 16.00 | 7.02 |
| $ZnF_2$ | 1.55 | 0.38 | 13.35 | 7.61 | 15.90 | 7.99 |
| $UO_2(NO_3)_2.6H_2O$ | N.S.R. | N.S.R. | 8.25 | 1.62 | 8.25 | 1.62 |
| $CuF_2$ | 3.00 | 0.70 | 12.50 | 8.37 | 15.50 | 9.07 |
| $KIO_3$ | 4.25 | N.S.R. | 12.50 | 7.75 | 16.75 | 7.75 |
| $CrF_2$ | 1.80 | N.S.R. | 12.30 | 5.54 | 14.10 | 5.54 |
| $BF_3$ | 0.15 | N.S.R. | 14.35 | 8.20 | 14.50 | 8.20 |
| $NH_4HF_2$ | 1.80 | 3.16 | 9.50 | 7.80 | 11.30 | 10.96 |
| $NH_4I$ | 5.75 | N.S.R. | 24.75 | 8.92 | 30.50 | 8.92 |
| $UO_2(AC).2H_2O$ | 4.90 | N.S.R. | 14.30 | 4.80 | 19.20 | 4.80 |
| $Na_2C_2O_4$ | * | 1.29 | * | 8.24 | | 9.53 |
| $(NH_4)_2C_2O_4$ | 7.45* | 2.50 | 10.15* | 6.40 | 17.60 | 8.90 |
| $K_2C_2O_4.H_2O$ | * | 1.92 | * | 8.20 | | 10.12 |
| $(COOH)_2.2H_2O$ | * | 1.50 | * | 7.86 | | 9.36 |
| $MgF_2$(H) | † | N.S.R. | † | 8.86 | | 8.86 |
| $SnCl_2$ | 1.05 | N.S.R. | 2.45 | 1.59 | 3.50 | 1.59 |
| KF | 0.60 | 0.46 | 16.90 | 8.70 | 17.50 | 9.16 |
| LiF | 0.50 | 0.21 | 15.20 | 7.61 | 15.70 | 7.82 |
| $NH_4F$ | 0.75 | N.S.R. | 17.55 | 8.53 | 18.30 | 8.53 |
| $NaHF_2$(H) | † | 0.38 | † | 4.85 | | 5.22 |
| KF(H) | 0.30 | N.S.R. | 16.90 | 7.88 | 17.20 | 7.88 |
| $CdF_2$(H) | 0.90 | N.S.R. | 7.90 | 3.23 | 8.80 | 3.23 |
| $CuF_2$(H) | 0.40 | N.S.R. | 12.60 | 5.72 | 13.00 | 5.72 |
| $SrF_2$(H) | † | N.S.R. | † | 7.53 | | 7.53 |

N.S.R. Not sufficient recovery.
*Interference was noted. Calculated directly from P data.
†Interference was noted. Calculated directly from Ca/P ratio.

reagent with a total loss of 7.49 mg. This is followed by stannous chloride with a total loss of 7.76 mg. The solubility for unprotected dentin is 21.65 mg.

For tricalcium phosphate the most effective solubility reducing compound is stannous fluoride I, with a solubility sum of 2.30 mg. This agrees with the sums obtained for enamel and dentin in that this compound is the most effective for both of these tissues and also for tricalcium phosphate. It should be noted that when the values are compared, less Ca and P are in solution for $Ca_3(PO_4)_2$ than for either enamel or dentin. The other five reagents also reduce the solubility of tricalcium phosphate in the following order of effectiveness: stannous chloride is second best with a solubility sum of 5.09 mg.; uranyl nitrate with a solubility sum of 9.87 mg. is third best; stannous fluoride II with a solubility sum of 11.03 mg. is fourth; cadmium fluoride with a solubility sum of 12.03 mg. is in fifth place; and stannous fluoride III with a solubility sum of 13.45 mg. is the least effective of this group.

TABLE V

COMPARISON OF MOST EFFECTIVE REAGENTS USED TO REDUCE THE SOLUBILITY OF ENAMEL, DENTIN, AND TRICALCIUM PHOSPHATE, SHOWING THE REAGENTS AND SUMS OF CA AND P FOUND IN FILTRATES

| REAGENT | ENAMEL FILTRATE CA & P (MG.) | REAGENT | DENTIN FILTRATE CA & P (MG.) | REAGENT | TRICALCIUM PHOSPHATE FILTRATE CA & P (MG.) |
|---|---|---|---|---|---|
| $SnF_2$ (I) | 3.25 | $SnF_2$ (I) | 4.65 | $SnF_2$ (I) | 2.10 |
| | 1.80 | | 1.90 | | 0.20 |
| | 5.05 | | 6.55 | | 2.30 |
| $SnF_2$ (II) | 3.55 | $SnF_2$ (II) | 4.70 | $SnCl_2$ | 3.50 |
| | 1.90 | | 2.03 | | 1.59 |
| | 5.45 | | 6.73 | | 5.09 |
| $Pb(NO_3)_2$ | 3.90 | $SnF_2$ (III) | 4.56 | $UO_2(NO_3)_2.6H_2O$ | 8.25 |
| | 2.40 | | 2.45 | | 1.62 |
| | 6.30 | | 7.01 | | 9.87 |
| $SnCl_2$ | 4.45 | $Pb(NO_3)_2$ | 5.60 | $SnF_2$ (II) | 8.00 |
| | 2.10 | | 1.89 | | 3.03 |
| | 6.55 | | 7.49 | | 11.03 |
| $SnF_2$ (III) | 4.70 | $SnCl_2$ | 5.08 | $CdF_2$ | 8.80 |
| | 2.50 | | 2.68 | | 3.23 |
| | 7.20 | | 7.76 | | 12.03 |
| $PbF_2$ | 4.82 | $PbF_2$ | 6.80 | $SnF_2$ (III) | 7.75 |
| | 2.44 | | 1.20 | | 5.70 |
| | 7.26 | | 8.00 | | 13.45 |
| NaF | 8.31 | NaF | 8.64 | NaF | 15.00 |
| | 4.28 | | 3.28 | | 3.25 |
| | 12.59 | | 11.92 | | 18.25 |
| $CH_3COOH$ | 12.70 | $CH_3COOH$ | 14.75 | $CH_3COOH$ | 20.13 |
| | 6.00 | | 6.90 | | 9.99 |
| | 18.70 | | 21.65 | | 30.12 |

It is evident, therefore, that stannous fluoride I is the only reagent that has the ability to reduce the solubility of enamel, dentin, and tricalcium phosphate with relative similarity. Stannous fluoride I is by far the best compound to use in reducing the solubility of enamel, dentin, and tricalcium phosphate. Less Ca and P is removed when they are treated with this salt than with any other protecting reagent so far observed.

## DISCUSSION

The objective of this investigation was to establish a method for describing more clearly than it has been done previously what takes place when unprotected and protected powdered enamel, dentin, and tricalcium phosphate are treated with acid. The data in Tables II, III, and IV show that when powdered enamel, dentin, and tricalcium phosphate are treated with acetic acid under the conditions of this experiment, Ca and P are removed and that the process is, therefore, truly one of decalcification. The data also demonstrate that certain reagents always reduce the amounts of Ca but not necessarily the P lost from powdered enamel when it is treated with acetic acid. This study shows that with respect to removal of Ca and P enamel is more resistant than dentin and dentin is more resistant than tricalcium phosphate.

It is further interesting to observe that some compounds not containing fluoride have again shown that they have the property of reducing the solubility of enamel and dentin. In this respect stannous chloride is the only one that appears consistently in the list of the first six most effective compounds. It is effective in reducing the solubility of both enamel and dentin as well as that of tricalcium phosphate. It is here suggested that it is not mere coincidence that the cation in each most effective salt is tin.

Early studies dealing with the solubility of enamel and dentin were investigated by measuring weight changes in the samples.[5, 6, 7] According to that procedure, a definite amount of enamel was placed in a suitable crucible and this tissue treated with the respective reagents. After sufficient time, the liquid phase was removed. This was then called the protected enamel sample. The enamel was then treated with an acetate buffer, pH 4.0, the crucible and contents dried and reweighed. The change in weight of this protected sample was compared with that of a decalcified unprotected powdered enamel sample. The difference in weight loss of the unprotected and protected enamel was taken as an indication of the reagent's ability to decrease the solubility of this tissue.

TABLE VI

SHOWING COMPARATIVE WEIGHT LOSSES BETWEEN VOLUMETRIC AND GRAVIMETRIC PROCEDURES IN MEASURING ENAMEL SOLUBILITY

| REAGENTS | ENAMEL FILTRATE CA & P (MG.) | WEIGHT CHANGE (MG.) |
|---|---|---|
| $SnF_2$ (I) | 3.25<br>1.80<br>5.05 | 0.3 |
| $SnF_2$ (II) | 3.55<br>1.90<br>5.45 | 3.2 |
| $Pb(NO_3)_2$ | 3.90<br>2.40<br>6.30 | 19.2 |
| $SnCl_2$ | 4.45<br>2.10<br>6.55 | 8.8 |
| $SnF_2$ (III) | 4.70<br>2.50<br>7.20 | 0.3 |
| $PbF_2$ | 4.82<br>2.44<br>7.26 | 10.2 |
| NaF | 8.31<br>4.28<br>12.59 | 10.1 |
| $CH_3COOH$ | 12.70<br>6.00<br>18.70 | 41.2 |

One can see in Table VI data with which to compare the findings obtained from the two different methods for studying tooth tissue solubility that have been used in this laboratory. The reagents are listed in column 1, the amounts

of Ca and P found in the filtrates in column 2, and the weight changes which occurred when the weight change method was used are seen in column 3. Only the values for enamel are shown, but similar studies were conducted for both dentin and tricalcium phosphate.

Examination of the data obtained by using the enamel weight change technic for unprotected enamel shows in the bottom row of Table VI column 3 a weight loss value of 41.2 mg. out of 100 mg. when treated with the acetic acid for 40 minutes. Using the technic in which the filtrate is examined for Ca and P removed from the unprotected tissue, the total amount lost was 18.7 mg. Comparing this value with stannous fluoride I, one finds a weight loss of 0.3 mg. on the one hand and a total loss of Ca and P amounting to 50.5 mg. on the other. These values show that stannous fluoride I is the best compound for protecting enamel in vitro. This is borne out both in the studies of weight change and loss of Ca and P. The value for the stannous chloride-treated enamel weight loss is 8.8 mg. However, when the Ca and P removed is measured, this salt is only the fourth most effective reagent with a total of 6.55 mg.

Many other differences can be discovered by closely examining this table, but suffice it to say that one should not rely in the future on a weight loss technic to evaluate the effectiveness of reagent solutions used to decrease the solubility of enamel and dentin. There is no doubt that the measurement of filtrate Ca and P is a better and more descriptive method than that of the former.

## SUMMARY

1. A method is presented whereby filtrates from enamel, dentin, and tricalcium phosphate obtained by treatment with reagent solutions and decalcified at pH 4.0 with an acetate buffer were analyzed for Ca and P.

2. Stannous fluoride was shown to decrease the solubility of enamel, dentin, and $Ca_3(PO_4)_2$ to the greatest extent.

3. Certain reagents not containing fluorine have the ability to reduce the solubility of enamel, dentin, and $Ca_3(PO_4)_2$.

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# OBSERVATIONS ON EXPERIMENTAL DENTAL CARIES

## X. Difference of Sex in Caries Susceptibility?

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THE clinical observations on sex difference in relation to caries susceptibility show that such a variation depends upon various factors. Several workers[1-5] have observed that girls exhibit a higher caries incidence than boys of the same age. This has been explained by Klein and Palmer[6] as due mainly to the fact that permanent teeth of girls erupt earlier, and, therefore, have been exposed to the oral environment for a longer period of time. The studies of Sloman,[7] however, do not agree with this explanation. On the other hand, some investigators[3, 4] have found higher caries incidence in the deciduous teeth of boys than of girls. More recently Burrill,[8] by means of bacterial and chemical determinations, studied the caries susceptibility of a large number of dental patients, and considered the results with reference to the age and sex of the subjects. According to the bacterial determinations he found that between 13 and 18 years of age males are more susceptible than females, and that between 19 and 21 years females are more susceptible than males. On the other hand, his chemical determinations showed that between 13 and 15 years of age females are more susceptible than males, between 16 and 18 years susceptibility is about the same in both sexes, and between 19 and 21 years females are less susceptible than males. These investigations were inconclusive since the bacterial and chemical results did not agree as to the time when peaks of susceptibility occur.

In albino rats Hunt and Hoppert[9] found only a slight indication that femaleness may sometimes be a minor factor in resistance to caries. On the other hand, Hodge[10] found no difference between caries incidence of normal and castrate female rats reared from the age of 1 year on a caries-producing diet for 19 months. In the Syrian hamster most North American workers[1-20] have reported, in general, higher caries susceptibility of males than females. Furthermore, Keyes[9] has reported a significant reduction of caries activity in both sexes, though more pronounced in males, as a consequence of gonadectomy. We, on the other hand, in our preceding studies on caries in hamsters[1-19] have found no consistently higher incidence of caries in males which could justify a positive statement on sex difference. Due to these contradictory findings in the same animal, and since there has been no reported studies based upon experiments carried out mainly with the purpose of studying such a suspected sex difference in caries susceptibility, we have carried out the following experiment, using a relatively large number of hamsters.

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## EXPERIMENTAL

Sixty-four newly weaned hamsters, from litters of a colony maintained on Purina Laboratory Chow* and raw milk, were litter-mate distributed into two groups as follows: Group 1, 32 males, and Group 2, 32 females. Both groups were reared for 100 days on the following diet: finely ground yellow corn 25 per cent, sucrose 25 per cent, cornstarch 20 per cent, powdered whole milk 22 per cent, ether-extracted yeast 5 per cent, alfalfa meal 2 per cent, salt mixture† 1 per cent, and d,l-α-tocopherol acetate 0.010 per cent. The diet, as well as tap water, were available ad libitum.

On completion of the experimental period the animals were sacrificed and autopsied. The molars were prepared for examination and the carious lesions were recorded and scored as previously.[1]

## RESULTS

Table I shows the caries activity of the two groups. It is clear that essentially the same incidence and extent of carious lesions were found in both groups. Therefore, under the conditions of this laboratory, the Syrian hamster does not exhibit any consistent and significant difference of sex with respect to dental caries susceptibility. The present studies confirm our previous observations on this subject.

TABLE I

CARIES ACTIVITY IN MALE AND FEMALE HAMSTER GROUPS

| | GROUP 1 ♂♂ | GROUP 2 ♀♀ |
|---|---|---|
| Percentage of animals affected | 96 | 100 |
| Average number of carious molars | 6.9 | 7.0 |
| Average number of carious lesions | 9.3 | 8.7 |
| Average caries scores | 5.6 | 4.9 |

The results of the present experiment, as well as our previous observations on the subject in this laboratory[1-19] do not agree with the findings of the North American workers.[1-20] Some of them[2, 3, 5, 9] have consistently found higher, and in some instances significantly greater, caries susceptibility of males than females when the hamsters have been reared on low or moderately caries-producing diets. On the other hand, we have formed no sex difference in caries susceptibility using precisely this kind of rations. Thus, an explanation for these contradictory findings on a sex difference in caries susceptibility of the hamster may lie on the existence of strain differences with respect to a sex-specific difference in dental caries susceptibility.

## SUMMARY

The difference of sex in caries susceptibility of the hamster has been studied using litter-mate, newly weaned animals for a 100-day experimental period. The results show that under the conditions of this laboratory the Syrian hamster

*From Ralston Purina Co., St. Louis, Mo., U.S.A.

†The salt mixture used was McCollum's Salt Mixture No. 185, supplemented with 13.5 mg.KI, 139 mg.$CuSO_4$, $5H_2O$, and 556 mg.$MnSO_4$, $4H_2O$ per 100 Gm.

does not exhibit difference of sex with respect to dental caries susceptibility. The present findings confirm our previous observations on this subject. The opposing results of these experiments as compared with the observations on the same subject made by other investigators have been pointed out.

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# THE POSTNATAL DEVELOPMENT OF THE MOLAR TEETH IN THE COTTON RAT

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THIS investigation was conducted to determine the rates of development and calcification and the eruption times of the individual molar teeth in specific strains of cotton rats. An accurate evaluation of these phases of dental growth and maturation was needed in the cotton rat as an adequate foundation for the interpretation of experiments to study the effects of prenatal and postnatal diets on tooth development and on the susceptibility of individual molar teeth to dental caries.

Data on the development of the molar teeth of the white rat and of the Syrian hamster have been presented by different investigators, but no previous study of the developmental pattern of cotton rat molars is known to exist. The eruption and calcification times of white rat molars have been determined under varying experimental procedures by Addison and Appleton,[1] Glasstone,[2] Mellanby,[3] Hoffman and Schour,[4] and Applebaum.[5] Orland[6] studied the chronological development of the molar teeth in the Syrian hamster by the use of radiographs and histologic sections.

Since two subspecies of cotton rat, *Sigmondon hispidus hispidus* and *Sigmondon hispidus littoralis*, are used in this laboratory for investigations on dental caries, representatives of both were selected for this study.

## MATERIALS AND METHODS

Cotton rats of subspecies, *Sigmondon hispidus hispidus*, were obtained from two sources as a means of comparing the dental development of offspring from two different stock colonies. A series of litters comprising 150 animals was selected from representative breeding pairs of our own colony. The time of birth of each litter was known within 12 hours. Until weaning time, the parents and the offspring were housed in cages with wood shavings as bedding and were offered stock ration and water ad libitum. The percentage composition of the stock ration was: Wilson's Ideal Dog Food V. T. 71.5 per cent, cracked corn 11 per cent, crude casein 7 per cent, alfalfa meal 7 per cent, and corn oil 3.5 per cent. After weaning, the offspring were placed in cages with wire bottoms but no change was made in the dietary regimen. A second series of litters of this subspecies totalling 103 animals was purchased from the Bureau of Laboratories of the Michigan Department of Health. Immediately upon weaning, these cotton rats were shipped according to litters and marked according to birth date. On arrival in this laboratory, they were placed in wire-bottomed cages and offered stock ration and water ad libitum. Ten cotton rats of a second

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subspecies, *Sigmondon hispidus littoralis,* were purchased from the Michigan Department of Health to be used for a rough estimation of the important times of dental development for this subspecies.

Individual animals were sacrificed at ages ranging from birth to 120 days. The first few litters used in this study were sacrificed at uniform, relatively wide intervals throughout this period to estimate the times of greatest activity in the development of the dental tissues. The remaining litters were sacrificed at appropriate times so that the greatest concentration of animals would be at the times of the important developmental processes in the molars. An average of 5 animals was sacrificed each day from birth to 20 days of age, from 26 to 36 days of age, and from 48 to 54 days of age. One or two animals were killed on each other day between these periods. Above 53 days of age, one cotton rat was sacrificed on about every tenth day to determine approximately the times at which calcification of the roots was complete. Males and females were represented as equally as possible in each period of the developmental pattern. At the time each animal was sacrificed, the sex and weight, birth date, parentage, and size of litter from which it was taken were recorded. The heads were fixed in 10 per cent formalin for at least 48 hours before removal of the major amount of skin and muscle tissue. Then, the mandibles were separated from the skull and the maxilla and mandibles divided into right and left quadrants.

Evaluation of the state of eruption of each molar was made by the examination of each quadrant with the aid of a binocular dissecting microscope using a magnification of 10.5 diameters. The degree of eruption of each molar was recorded according to the following criteria: (a) initial eruption—when the tip of a cusp had pierced the oral mucosa; (b) early, partial eruption—when part of the crown had emerged; (c) late partial eruption—when most of the crown had penetrated the mucosa; and (d) complete eruption—when the entire anatomical crown had penetrated the mucosa.

All four quadrants were then radiographed using a Ritter machine with 10 milliamperes' current for 4 seconds at 20 inches. The plate used to record the image was a Kodak lantern slide with emulsion of medium contrast; by the use of this procedure, resulting radiographs could be studied at the necessary magnification of 10.5 diameters without distortion due to the larger grains which compose the emulsion of the usual grades of x-ray film. The extent of calcification of the crown and root of each molar tooth was determined and recorded.

The left or right quadrants of 16 cotton rats which had been selected as representative of the group of animals sacrificed at each of 16 different developmental periods were studied after histologic preparation. The quadrants were decalcified by the formic acid-citrate method described by Morse,[7] dehydrated, and then imbedded in paraffin. Serial sections were made and stained with hematoxylin and eosin. These sections were then studied to elaborate upon the data obtained by examination of the intact teeth and their radiographs.

While the various dental developmental studies were being evaluated for cotton rats fed the stock ration, casual observations of the state of eruption of the molars at the end of certain experiments indicated that the development of the third maxillary and mandibular molars in cotton rats fed purified rations[8]

differed from comparable rats fed the stock ration. Numerous cotton rats which were killed after 14 weeks on experiment, that is, at between 16 and 17 weeks of age, did not have completely erupted third molars and, in some cases, the cusps of the third molars had not pierced the mucosa. This difference suggested the advisability of collecting and summarizing data on the eruption of third molars in cotton rats fed the purified ration for various periods after they were weaned, prior to which time they, and their parents, had had access only to the stock ration. For this purpose, data were collected for 1,106 cotton rats of the subspecies, *Sigmondon hispidus hispidus*, between 36 and 120 days of age which were not maintained specifically for this purpose but for a variety of experiments concerned with the susceptibility to tooth decay. Insufficient numbers of cotton rats were available on any purified ration to permit summary of the data on the basis of an individual diet. All rations were adequate in so far as their vitamin, minerals, and protein content were concerned. No detailed histologic studies of the third molars have been made of the cotton rats maintained on the purified rations during tooth development.

## RESULTS AND DISCUSSION

The data on the calcification and eruption times for the molar teeth of cotton rats of the subspecies, *Sigmondon hispidus hispidus*, fed the stock ration throughout the experimental periods, are presented in Tables I and II. Since there was no detectable difference in the dental developmental times between the representatives of this subspecies born in our stock colony and those born in the colony at the Bureau of Laboratories of the Michigan Department of Health, the data obtained with the 150 animals from the former source and those with the 103 from the latter source were considered together in the preparation of both tables. The results on tooth development are tabulated in Table I as a function of the age in days after birth. Since there was a very considerable variation in the age at which the various developmental stages were observed to occur, a range is given for each activity from the earliest to the latest day at which the criteria for that activity were satisfied. In view of the possibility that the body weight of the animals at the time of sacrifice might be a more accurate base line for correlation with dental development, the data were also tabulated according to final body weight (Table II). Again a rather wide variation was observed and recorded in the same manner as in Table I. In general, age and weight were equally satisfactory criteria to which dental development in cotton rats could be related. The times of various stages of tooth formation did not appear to vary between sexes.

The range of variation in eruption times can be illustrated best by a description of the observations recorded for any one tooth. In the case of the first maxillary molar, no evidence of emergence into the oral cavity was observed in 6 cotton rats sacrificed on the fourth day. The initial emergence of one cusp through the mucosa was observed on the fifth day in one of 5 cotton rats; in the remaining 4, there was no evidence of perforation of the mucosa. On the sixth day, 2 animals of 5 showed an initial eruption of the first maxillary molar, one presented late partial eruption, and in 2 there was no evidence of eruption into

the oral cavity. On the seventh day post partum, one cotton rat presented an initial stage of emergence of the first maxillary molar, one showed early partial eruption, and 3 late partial eruption. On the eighth day, the first maxillary molars of 2 animals were judged to be in a state of early partial eruption, 2 in late partial eruption, and one completely erupted. By the twelfth postnatal day, all first maxillary molars were fully erupted. This variation in eruption of the first maxillary molars was characteristic of the variation in eruption of the other molars. In all stages of dental development, roughly the same degrees of variation were observed. Variation among littermates still existed but was somewhat less than for random cotton rats.

TABLE I

CALCIFICATION AND ERUPTION TIMES FOR THE MOLAR TEETH OF THE COTTON RAT (SIGMONDON HISPIDUS HISPIDUS)
(Figures are expressed in days postnatally)

| | MAXILLARY MOLARS | | | MANDIBULAR MOLARS | | |
|---|---|---|---|---|---|---|
| | FIRST | SECOND | THIRD | FIRST | SECOND | THIRD |
| First appearance of dental lamina | Before birth | Before birth | 1 to 3 | Before birth | Before birth | At birth to 2 |
| Beginning of calcification | Before birth | Before birth to 3 | 9 to 14 | Before birth | Before birth to 2 | 8 to 12 |
| Completion of appositional growth in crown | 3 to 6 | 7 to 13 | 26 to 34 | 3 to 5 | 6 to 11 | 24 to 30 |
| Beginning of bifurcation of roots | 6 to 9 | 14 to 18 | 35 to 40 | 4 to 8 | 12 to 18 | 33 to 40 |
| First appearance in oral cavity | 5 to 7 | 9 to 13 | 28 to 33 | 4 to 7 | 7 to 12 | 26 to 30 |
| Complete eruption | 7 to 12 | 15 to 20 | 40 to 54 | 6 to 10 | 12 to 19 | 31 to 42 |
| Root completely formed | 25 to 32 | 50 to 70 | 90 to 110 | 22 to 28 | 45 to 60 | 70 to 90 |

TABLE II

WEIGHTS OF COTTON RATS (SIGMONDON HISPIDUS HISPIDUS) AT WHICH CALCIFICATION AND ERUPTION OF MOLAR TEETH OCCURRED
(Figures are expressed in grams)

| | MAXILLARY MOLARS | | | MANDIBULAR MOLARS | | |
|---|---|---|---|---|---|---|
| | FIRST | SECOND | THIRD | FIRST | SECOND | THIRD |
| First appearance of dental lamina | Before birth | Before birth | 4 to 8 | Before birth | Before birth | 4 to 7 |
| Beginning of calcification | Before birth | Before birth to 9 | 14 to 30 | Before birth | Before birth to 7 | 12 to 26 |
| Completion of appositional growth in crown | 7 to 15 | 12 to 23 | 32 to 48 | 7 to 12 | 11 to 23 | 30 to 45 |
| Beginning of bifurcation | 11 to 18 | 25 to 36 | 42 to 60 | 9 to 15 | 20 to 33 | 35 to 55 |
| First appearance in oral cavity | 9 to 16 | 16 to 23 | 35 to 51 | 8 to 14 | 15 to 24 | 30 to 45 |
| Complete eruption | 15 to 24 | 26 to 36 | 49 to 76 | 14 to 21 | 22 to 36 | 35 to 58 |
| Root completely formed | 30 to 50 | 55 to 79 | 90 to 120 | 27 to 46 | 48 to 73 | 72 to 90 |

The modes of emergence of the mandibular molars into the oral cavity were 5 days of age for the first molar, 9 days for the second molar, and 28 days for the third molar. The modes of emergence of the maxillary molars into the oral cavity were 6 days of age for the first molar, 11 days for the second molar, and 31 days for the third molar. In all phases of tooth formation, the maxillary

molars were slightly later than the mandibular molars. This length of time increased from about one day for the first molars to 3 or 4 days for the third molars of cotton rats fed diets composed of natural foodstuffs.

Fig. 1.

Fig. 2.

Fig. 1.—Radiograph of right half of skull of one-day-old cotton rat. Calcification of crowns of first molars is well advanced; calcification of second molar crowns is just beginning. No radiographic evidence of third molars. (×4)

Fig. 2.—Radiograph of right half of skull of 14-day-old cotton rat. First molars are fully erupted, second molars are almost fully erupted, while crowns of third molars have just begun to calcify. (×4)

At birth, the first molars of cotton rats are in an advanced stage of calcification; the second molars have begun to calcify and the buds of the third molars are just being differentiated (Figs. 1, 5, 6). Experimental procedures begun at this stage would have little or no effect on crown formation of the first molars but could influence the later stages of development in the second molars and the entire life of the third molars. As yet no studies have been conducted in this

laboratory with the cotton rat beginning at birth to determine the influences of any diet other than stock ration or any dietary supplement on the development of teeth.

Fig. 3.

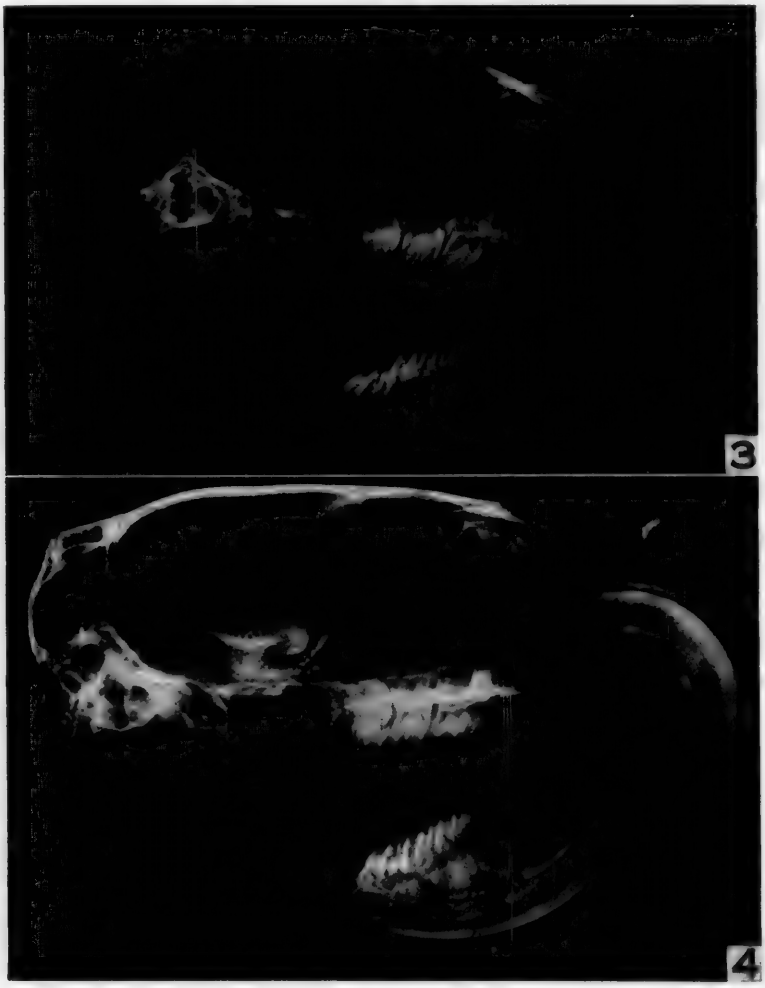


Fig. 4.

Fig. 3.—Radiograph of right half of skull of 31-day-old cotton rat. First and second molars are fully erupted, while crowns of third molars have just begun to erupt. Root formation appears to be almost complete in first molars and well advanced in second molars. (×4)

Fig. 4.—Radiograph of right half of skull of 120-day-old cotton rat. Tooth development appears to have been completed. (×4)

Cotton rats are normally weaned at 14 days of age. The dental development of weanling cotton rats is of particular interest in view of the fact that this is one of the earliest ages at which controlled experimental regimens can be begun. At this age, the first molars were fully erupted and the second molars have just

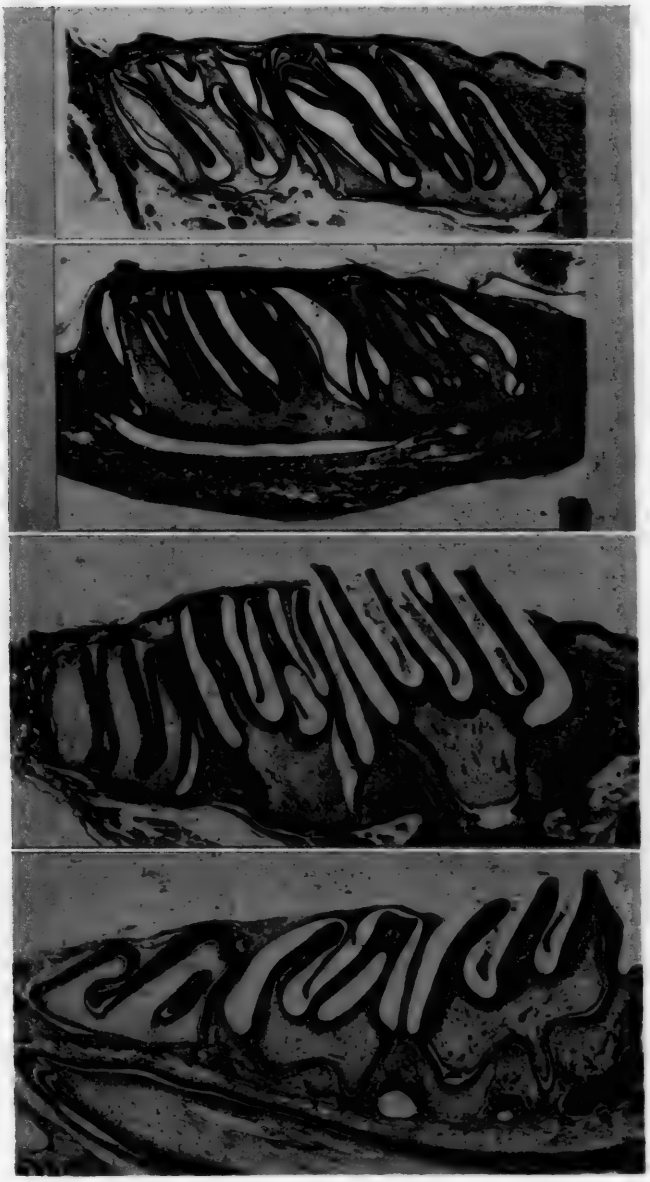

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Figs. 5 to 8.—Photomicrographs of histologic sections made from decalcified preparations which were sectioned at 10 microns and stained with hematoxylin and eosin. (Magnification ×18)

Fig. 5.—Mesiodistal section from right maxilla of one-day-old cotton rat skull radiographed for Fig. 1.

Fig. 6.—Mesiodistal section from right mandible of one-day-old cotton rat skull radiographed for Fig. 1.

Fig. 7.—Mesiodistal section from right maxilla of 14-day-old cotton rat skull radiographed for Fig. 2.

Fig. 8.—Mesiodistal section from left mandible of 14-day-old cotton rat skull radiographed for Fig. 2.

begun to erupt. The crowns of the third molars are in an early stage of formation and calcification (Figs. 2, 7, 8). Thus, any experimental procedure begun at this age would be expected to only influence root formation in the first and second molars, but could influence crown formation, eruption, and root formation of the third molars.

At 31 days post partum, crown formation in the molars of the cotton rat is largely completed, the first and second molar teeth are fully erupted, and the third molars in most animals have emerged into the oral cavity (Figs. 3, 9, 10). In experiments where it is desired to have no influence of a diet other than the stock ration pre-eruptively, the 31-day-old cotton rat is about the youngest representative which can be used. Experimental variations in the diet begun much before that age could influence development of the third molars to some extent.

The 10 representatives of a second subspecies *Sigmondon hispidus littoralis* were found to have times of dental development which in all cases fell within the ranges observed for representatives of the subspecies *Sigmondon hispidus hispidus*. The small number of cotton rats from the former subspecies was insufficient to permit the detection of any small deviations which might exist.

The survey of the state of eruption for the third molars of cotton rats fed purified rations for various periods after weaning at 14 days of age is presented in Table III. In all age groups, from 36 to 120 days post partum, the third molars appeared to have been appreciably delayed in their emergence into the oral cavity and in the attainment of complete eruption. For example, in all cotton rats maintained until 42 days of age on the natural stock ration, the mandibular third molars were fully erupted. Of the 14 cotton rats maintained on purified rations from weaning until 41 to 45 days of age, the mandibular third molars were fully erupted in 9 animals, partially erupted in 2, and had not pierced the mucosa in 3. In 854 cotton rats fed purified diets from weaning until 116 to 120 days old, the lower third molars were only partially erupted in 49 cases and had not emerged into the oral cavity in an additional 13 cases. A still more delayed state of eruption was observed for the maxillary third molars. In all cotton rats maintained until 54 days of age on the stock ration, the maxillary third molars were fully erupted and in occlusion. Among 11 cotton rats fed purified rations after weaning until 51 to 55 days of age, the maxillary third molars were fully erupted in 7 animals, partially erupted in 3, and had not penetrated through the mucosa in one. Among the 854 cotton rats fed purified ration until 116 to 120 days of age, the upper third molars were partially erupted in 81 animals and had not emerged into the oral cavity in an additional 77 cases. The groups of cotton rats in between the age groups discussed above demonstrated the same retardation in rate of dental development.

The above summary on the effect of purified rations on the time of emergence and rate of eruption of third molars indicates that there has been definitely slower rates of dental development in the cotton rat. If the rates of tooth formation and eruption in cotton rats fed stock ration composed of natural foodstuffs could be considered as normal, maintenance on the purified rations would have to be considered as having caused abnormally retarded rates of development

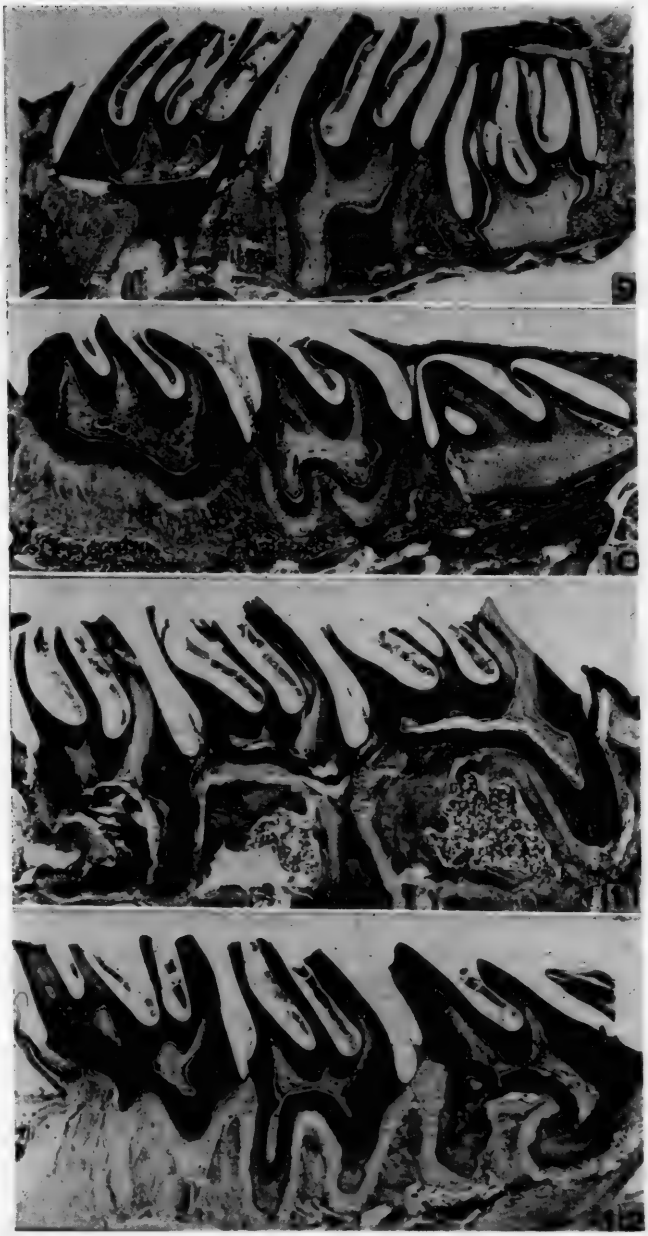

Fig. 9.

Fig. 10.

Fig. 11.

Fig. 12.

Figs. 9 to 12.—Photomicrographs of histologic sections made from decalcified preparations which were sectioned at 10 microns and stained with hematoxylin and eosin. (Magnification ×18.)

Fig. 9.—Mesiodistal section from left maxilla of 31-day-old cotton rat skull radiographed for Fig. 3.

Fig. 10.—Mesiodistal section from right mandible of 31-day-old cotton rat skull radiographed for Fig. 3.

Fig. 11.—Mesiodistal section from right maxilla of 120-day-old cotton rat skull radiographed for Fig. 4.

Fig. 12.—Mesiodistal section from right mandible of 120-day-old cotton rat skull radiographed for Fig. 4.

TABLE III

SUMMARY OF STATE OF ERUPTION OF MAXILLARY AND MANDIBULAR MOLARS OF COTTON RATS FED PURIFIED RATIONS FOR VARYING PERIODS AFTER WEANING

| POSTNATAL AGE (IN DAYS) | NUMBER OF COTTON RATS | DEGREE OF ERUPTION | | | | | |
|---|---|---|---|---|---|---|---|
| | | MAXILLARY THIRD MOLAR | | | MANDIBULAR THIRD MOLAR | | |
| | | CUSPS NOT THROUGH MUCOSA | PARTIAL | COMPLETE | CUSPS NOT THROUGH MUCOSA | PARTIAL | COMPLETE |
| 36- 40 | 15 | 4 | 7 | 4 | 5 | 1 | 9 |
| 41- 45 | 14 | 3 | 6 | 5 | 3 | 2 | 9 |
| 46- 50 | 14 | 4 | 2 | 8 | 3 | 1 | 10 |
| 51- 55 | 11 | 1 | 3 | 7 | 0 | 0 | 11 |
| 56- 60 | 8 | 3 | 0 | 5 | 0 | 2 | 6 |
| 61- 65 | 13 | 5 | 0 | 8 | 2 | 1 | 10 |
| 66- 70 | 9 | 0 | 2 | 7 | 0 | 1 | 8 |
| 71- 75 | 21 | 6 | 4 | 11 | 2 | 3 | 16 |
| 76- 80 | 18 | 1 | 2 | 15 | 0 | 3 | 15 |
| 81- 85 | 12 | 5 | 2 | 5 | 2 | 3 | 7 |
| 86- 90 | 18 | 0 | 5 | 13 | 0 | 1 | 17 |
| 91- 95 | 26 | 3 | 3 | 20 | 0 | 3 | 23 |
| 96-100 | 21 | 2 | 8 | 11 | 0 | 2 | 19 |
| 101-105 | 21 | 1 | 9 | 11 | 1 | 2 | 18 |
| 106-110 | 14 | 1 | 3 | 10 | 0 | 1 | 13 |
| 111-115 | 17 | 3 | 4 | 10 | 0 | 2 | 15 |
| 116-120 | 854 | 77 | 81 | 696 | 13 | 49 | 792 |

and eruption. Genetic constitution was not likely concerned with these differences, since the offspring used in the studies with the purified rations were siblings of the 150 cotton rats obtained from our own stock colony for studies of rate of dental development. The differences in tooth formation and eruption involved between purified rations and the stock ration were sufficiently great to suggest considerable further investigation as to the nutritional cause, or causes, of the difference and the pathway through which they were mediated.

In addition to the above evidence of a suggested inadequacy of the purified rations as far as tooth development is concerned, observations that the purified rations fed during tooth development influence the susceptibility to tooth decay have been presented for the cotton rat, the common laboratory rat, the hamster, and the mouse. In the cotton rat, Shaw, Schweigert, McIntire, Elvehjem, and Phillips[9] observed that the third molars of cotton rats fed the purified rations from weaning at 14 days for an additional 14 weeks had a higher susceptibility to tooth decay than the first and second molars. This observation was found despite the fact that the third molars were not exposed through the oral environment to the purified rations for the full experimental periods due to their late eruption. Later experiments in this laboratory have indicated that third molars which were allowed to develop and erupt during maintenance on the stock ration did not have a higher susceptibility to tooth decay than the first and second molars. Likewise, Sognnaes[10] has shown that female rats, hamsters, and mice maintained on purified rations through the reproduction cycle bore offspring which had a higher susceptibility to dental caries than the offspring of comparable females fed laboratory chow as the stock ration throughout gestation and lactation.

The above observations on the inability of the purified rations to permit normal rates of tooth development and eruption, and on their inability to produce teeth of high caries resistance, suggest the need to evaluate the differences between the stock rations used in these experiments and the purified rations.

## SUMMARY

The rates of postnatal development and eruption for the molar teeth of cotton rats maintained on a stock ration were determined.

A survey was made on the effect of purified rations fed to cotton rats after weaning at 14 days of age upon the time of eruption of the maxillary and mandibular third molars. The time of emergence into the oral cavity and the completion of eruption of the third molars were much later in these cotton rats than in those maintained on stock rations.

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# DOES GLUCOASCORBIC ACID PRODUCE SCURVY?

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SINCE the original work of Woods (1940), many cases of action of antimetabolites have been investigated. Among these Woolley and Krampitz (1943) described the action of glucoascorbic acid† as leading to a "scurvy-like condition" in species which normally produce their own ascorbic acid. Banerjee and Elvehjem (1945) have repeated and somewhat extended Woolley's observations and while weight losses, diarrhea, etc., were recorded, they found no gross hemorrhage or significant change when extracts of liver and kidney were titrated for ascorbic acid. While the latter type of evidence is plausible enough, it is not any too convincing since the ascorbic acid cannot be titrated as such, the glucoascorbic which is administered having the same reducing action on the reagent.

Gross hemorrhage in experimental scurvy in guinea pigs can be considered subsequent to strain or trauma (Follis, 1948). Since chance for strain or trauma may be less in mice or rats than in guinea pigs and in view of other changes in experimental conditions, it seemed warranted to re-examine the question on the basis of histological examination which would reveal both more primary and also minor scorbutic changes. Follis (1948) has presented an excellent review of the pathogenesis of scurvy. The basic defect in scurvy is in the intercellular substances, of which osteoid material is an example. The most sensitive and unequivocal signs can be seen in the long bones and dentin. There is a failure of the osteoblasts to lay down osteoid material on the calcified cartilage spicules, and large numbers of osteoblasts accumulate in the marrow cavity. The relatively weak cartilage spicules, unencased in bone, remain and undergo fracture. If trauma is marked, areas of hemorrhage and numbers of macrophages are seen, but these are admittedly secondary manifestations, as are the fractures of the calcified cartilage spicules.

## METHODS

Sixteen Webster-Swiss mice, 21 days old, were used. Two males and 2 females from each litter were chosen and divided between the experimental and control groups, such that there were 8 experimental and 8 control animals, 4 in each group being male and 4 female. The animals were chosen so that for each group the average initial weight was 10 grams and no individual weighed more than 11 grams or less than 9 grams. The glucoascorbic acid composed 5 per cent of the diet by weight. It was added daily to minimize oxidation. At

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†Glucoascorbic acid is related to glucose as ascorbic acid is to xylose. Structurally it is: 2,3-enediol-d-glucohepteno-1,4-lactone.

the completion of the experiment, the glucoascorbic acid was checked by titration with 2,6 dichlorophenol-indophenol and found to have its original potency. A mouse does not eat more than 3 Gm. of the diet per day, at the age of 20 to 30 days, and therefore, fresh daily quantities of 3 Gm. each were given. Anorexia increased as the investigation proceeded. The mice were observed daily, clinical changes noted, and weight was recorded every other day.

A diet very similar to that utilized by others (Woolley and Krampitz, 1943, Banerjee and Elvehjem, 1945, Woolley, 1944) was used, 100 Gm. of basal diet containing: casein ("vitamin-test"), 24 Gm., sucrose, 64 Gm.; Crisco, 5 Gm.; cod liver oil, 3 Gm.; and salts, 4 Gm. (Hegsted, Mills, Elvehjem, and Hart, 1941). The fat-soluble vitamins, A and D, were provided in the cod liver oil incorporated in the diet. Sufficient quantities of the diet were made for a week's feeding and stored in a cool place. Water-soluble vitamin supplements were administered orally in 10 per cent sucrose solution three times a week in the following doses per animal per day; thiamine hydrochloride 20γ; pyridoxine hydrochloride 20γ; riboflavin 40γ; niacin 1 mg.; choline chloride 4 mg.; para-aminobenzoic acid 250γ; I-Inositol 500γ; vitamin E* 400γ; Vitamin K† 80γ; and calcium pantothenate 200γ.

## RESULTS

The control male and female mice gained 4.6 grams and 4.1 grams per week respectively at the end of two weeks, i.e., an average gain of 9.2 and 8.2 grams for the two weeks, while the males and females from the experimental group lost 1.9 and 2.1 grams respectively for the two-week interval.

The observations of others with respect to diarrhea, perianal inflammation, weight loss, and alopecia were confirmed in this study. However, on the twelfth day several of the animals exhibited a mild hemorrhagic diarrhea. At no time were subcutaneous hemorrhages or hematomas observed. The gingivae were not inflamed.

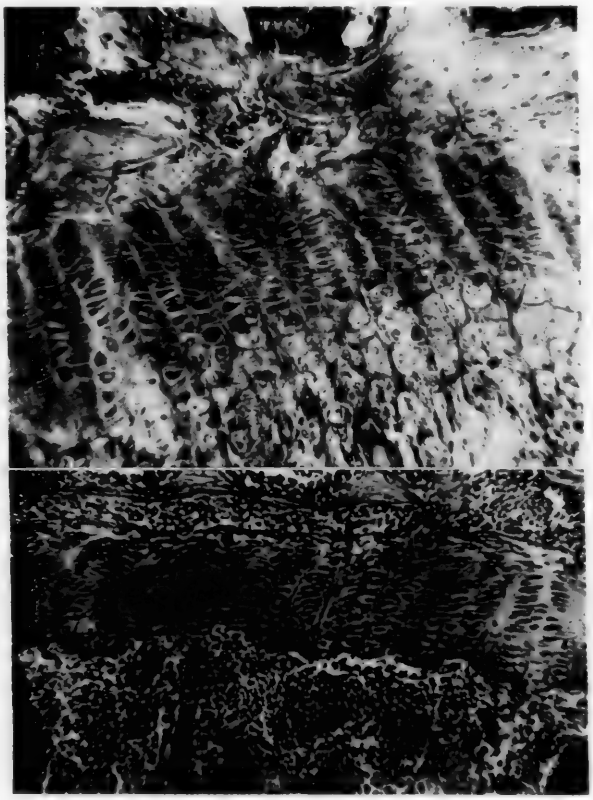
The diarrhea observed by all investigators suggests intestinal irritation. Banerjee and Elvehjem (1945) proposed that this may be due to a microbiological change in the intestinal flora. The rapid onset and further course rather speak for irritant properties in the glucoascorbic acid itself.

A slight change in the molecule of physiologically highly active substances such as vitamins can result in four possible effects: (a) a physiologically inactive substance may result; (b) a vitamer may be produced; (c) an antimetabolite may be formed; or (d) given a physiologically highly active molecule, a minor change in structure may result in another potent substance sharing none of the original biological properties nor their opposites, but having a new type of activity which may even be toxic. It appears that in the diarrhea production by glucoascorbic acid we are dealing with the fourth possibility. It is interesting to note that diarrhea is produced when ascorbic acid is substituted for glucoascorbic acid as shown by Banerjee and Elvehjem (1945).

*D-tocopherol, phosphoric acid, disodium, synthetic water-soluble vitamin E.
†Synkayvite-synthetic vitamin K, water-soluble.

Microscopic scorbutic criteria were not seen. Osteoid material was laid down upon the cartilage spicules in a normal manner and amount. There was no increase in the number of osteoblasts in the marrow cavity. However, there was a profound diminution in the number of proliferating cartilage cells in the epiphyseal plate, accompanied by a failure of maturation of these cells. The normal swelling and breakdown of the cartilage cells near the marrow cavity was not observed. Calcification of the cartilage and bone occurred to a lesser degree in the experimental group. The bone marrow appeared normal, but greater vascularity was noted. The changes in the bones were uniform throughout the experimental series and can be fully explained upon the basis of inanition.

*A.*



*B.*

Fig. 1.—Photomicrographs of epiphyseal plate of the proximal end of the tibia of normal and experimental mice.

*A*, Normal mouse. Note normal maturation of cartilage cells.

*B*, Experimental mouse. There is cessation of bone formation. The epiphyseal plate is markedly narrowed and no hypertrophic cartilage cells are observed. The intercellular cartilage matrix is increased in amount.

The teeth were observed for histopathologic changes. The characteristic changes seen in guinea pig scurvy, atrophy and disarrangement of the odonto-

blasts, irregular deposition of dentin, and hemorrhage into the pulp were all remarkably absent. The pulp was normal in appearance, having only an increased degree of vascularity as was observed in the bone marrow. The other organs were studied, but no changes other than those compatible with inanition, save a generalized increase in vascularity, were noteworthy.

The failure of glucoascorbic acid to produce a histopathological picture of scurvy in the bones of weanling mice adds further interest to the studies of Mayer and Krehl (1948) on the manifestation of the scorbutic state in vitamin A-deficient rats which also ordinarily synthesize their own ascorbic acid. Their work demonstrates that rats, at least, are capable of a scorbutic state.

Since this work has been completed, a paper has appeared (Gould 1948) dealing with the possibility of competition between ascorbic and glucoascorbic acids on the basis of serum phosphatase changes. Gould found no apparent antagonism existing between l-ascorbic acid and d-glucoascorbic acid, even when the latter was fed in massive amounts.*

## SUMMARY

The feeding of a purified diet containing 5 per cent glucoascorbic acid to weanling mice produced anorexia, mild alopecia, diarrhea, and weight loss. The histopathologic changes in the long bones and teeth were not compatible with the diagnosis of even initial scurvy. They resembled those changes ordinarily seen in inanition. The marrow of the bones and the pulp of the teeth as well as the viscera exhibited an increased vascularity.

The author wishes to express his thanks to Dr. Phillip Gray of the Wallerstein Laboratories and to Dr. Robert Ballentine of the Zoology Department, Columbia University, for generous gifts of glucoascorbic acid. In addition, the author wishes to thank Miss Marie Novello for her technical assistance in preparation of the histopathological sections.

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*An additional publication (Follis, R.: *Bull. Johns Hopkins Hosp.* **84**: 204, 1949) presents findings identical to those of the author.

# DENTAL CHANGES IN RATS ON A PURIFIED DIET DEFICIENT IN VITAMIN E

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## INTRODUCTION

AFTER Black, McKibbin, and Elvehjem[1] showed, in 1941, that the addition to the diet of sulfaguanidine inhibits the growth of certain microorganisms which will normally synthetize essential factors of the B complex, numerous experiments have been carried out in this field, and it has been found that most of the factors of the B complex and vitamin K may be synthetized in the gastrointestinal tract.

A study on the influence of folic acid and methylfolic acid on the blood picture of white rats fed a diet deficient in vitamin E and containing succinylsulfathiazole[2] showed some changes in the incisors which, in previous reports, have not been attributed to similar factors. In the present paper it is, therefore, proposed to give a preliminary report on the character of these changes.

## MATERIAL AND METHODS

The rats used in the experiments were 4-week-old white rats of the Wistar strain. The basal diet (F.I) had the composition shown in Table I.

TABLE I

COMPOSITION OF THE PURIFIED DIET (F.I)

| | | | |
|---|---|---|---|
| Glucose | 70 | Vitamin mixture: | |
| Casein, purified | 20 | Choline HCl | 1 Gm. |
| Salt mixture | 4 | Nicotinic acid | 50 mg. |
| Groundnut oil and 1600 I.U. Vitamin A and 150 I.U. Vitamin D | 3 | Thiamine HCl | 10 mg. |
| | | Riboflavin | 10 mg. |
| | | Ca pantothenate | 50 mg. |
| Succinylsulfathiazole | 1 | Pyridoxine HCl | 10 mg. |
| Vitamin mixture | 2 | P-aminobenzoic acid | 10 mg. |
| | 100 | Vitamin K | 5 mg. |
| | | Glucose to make | 20 Gm. |

In the experiments 39 rats, divided into five groups, were used. Group 1 received the basal diet (F.I); Group 2, F.I + 5 mg. folic acid per kg. of diet (F.II); Group 3, F.I + 1 Gm. methylfolic acid per kg. (F.III); Group 4, F.I + 5 mg. folic acid and 1 Gm. methylfolic acid (F.IV), and finally Group 5 was given F.I + 25 mg. folic acid and 1 Gm. methylfolic acid per kg. diet (F.V). All the experimental animals were given water ad libitum, and they were weighed once every week. In Group 3 only, a few rats died before the experimental period had expired. The remaining animals were killed after the experiment had lasted from 49 to 53 days. Blood was withdrawn for hematological study, and the teeth were examined.

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For the registration of the color of the incisors a color scale was made, comprising fourteen different colors numbered from 1 to 14. No. 1 was white and represented complete depigmentation; No. 14 was rather of the hue of Spanish mahogany, the most intensive pigmentation observed which was that of the incisors of the beaver rat. After registration of the colors the skulls were cut sagittally and roentgenograms made. After fixation in 10 per cent formalin the maxillae were decalcified in 5 per cent nitric acid and, after having been thoroughly washed and dehydrated, they were embedded in celloidin. Some few maxillae were embedded in paraffin. All sections were stained with hematoxylin and eosin.

## RESULTS

The changes observed in the teeth fall into two groups. First, clinically, a depigmentation of the incisors, which are normally orange-colored, was observed. Because of this finding a histologic examination of the incisors was made, which demonstrated a series of other characteristic histopathologic changes in the enamel organ.

### *1. Depigmentation of the Incisors*

Table II presents a survey of the material. Normally, the incisors of the upper and lower maxillae will be of Colors 10 and 7, respectively, registered according to the above-mentioned color scale. A consideration of the average color intensity within the five groups shows that a reduction has taken place in all cases. The most marked depigmentation is seen in Group 2, where the average is 4.4 for the upper incisors and 4.0 for the lower incisors. In the great majority of cases, depigmentation occurred first in the upper jaw and there was but rarely (only in No. 37) a complete depigmentation of the lower incisors. Typical of the onsetting depigmentation was a narrow longitudinal stripe in the upper incisors.

### *2. Histopathologic Changes in the Enamel Organ*

The enamel organ of the incisors of the white rat extends throughout the length of the tooth. Basally, an epithelial node (the odontogenetic epithelium) is found, from which ameloblasts and the so-called stratum papillare, which is composed of the stratum intermedium, stellate reticulum, and outer enamel epithelium of the enamel organ, and capillary invaginations from the surrounding connective tissue are continuously being differentiated. In the rat incisors, a physiologic atrophy of ameloblasts as well as of the stratum papillare takes place. The level of the onset of atrophy is dependent on age. In the very young rats the ameloblasts retain their typical structure all the way to the site of junction with the gingival epithelium. In a 3-month-old rat the atrophy occurs somewhat earlier, and the ameloblasts lose their original cylindrical form slightly earlier than does the stratum papillare, to assume a cubical form, finally to be united with the gingival epithelium. Also the stratum papillare is reduced, and fuses with the ameloblasts and the gingival epithelium. In Fig. 1 *1* and *2* indicate the points at which atrophy of the ameloblasts and the stratum papillare, respectively, occurs. The distance from the place where the enamel becomes acid soluble (*3*) to the place of the atrophy of the stratum papillare is 8.0 mm., in a straight line, in the maxilla of a 3-month-old rat.

TABLE II
COLOR CHANGES IN INCISORS

| NO. | DIET | AGE AT BEGINNING OF EXPERIMENT (DAYS) | DURATION OF EXPERIMENT (DAYS) | COLOR INTENSITY OF INCISORS | | HISTOLOGY OF UPPER INCISORS | DISTANCE FROM ENAMEL MATRIX TO PAPILLARY ATROPHY (MM.) | DIED OR KILLED |
|---|---|---|---|---|---|---|---|---|
| | | | | UPPER | LOWER | | | |
| 1 | Basal diet (F.I) | 36 | 53 | 10 | 7 | + | | k |
| 2 | | 36 | 53 | 9 | 7 | + | 2.9 | k |
| 3 | | 36 | 53 | 8 | 6 | ++ | 3.3 | k |
| 4 | | 35 | 53 | 2 | 6 | + | 3.6 | k |
| 5 | | 35 | 53 | 6 | 7 | ++ | 2.8 | k |
| 6 | | 35 | 53 | 9 | 6 | + | 3.7 | k |
| 7 | | 35 | 53 | 10 | 7 | + | 3.8 | k |
| 8 | | 35 | 53 | 7 | 7 | + | 3.4 | k |
| | Average color intensity of Group 1 | | | 7.6 | 6.6 | | | |
| 9 | F.I + 5 mg. folic | 35 | 52 | 1 | 2 | ++ | 4.1 | k |
| 10 | acid per kg. | 35 | 52 | 2 | 3 | ++ | 4.1 | k |
| 11 | | 35 | 52 | 5 | 6 | ++ | 3.6 | k |
| 12 | | 35 | 52 | 9 | 6 | ++ | | k |
| 13 | | 35 | 52 | 2 | 6 | ++ | | k |
| 14 | | 35 | 52 | 3 | 3 | + | | k |
| 15 | | 35 | 52 | 5 | 2 | ++ | 5.3 | k |
| 16 | | 35 | 52 | 8 | 5 | ++ | 4.3 | k |
| | Average color intensity of Group 2 | | | 4.4 | 4.0 | | | |
| 17 | F.I + 1 Gm. | 35 | 48 | 10 | 7 | + | | d |
| 19 | methylfolic | 35 | 39 | 4 | 3 | + | | d |
| 20 | acid per kg. | 35 | 49 | 9 | 6 | ++ | 5.7 | d |
| 21 | | 35 | 46 | 9 | 6 | + | | d |
| 22 | | 35 | 49 | 10 | 7 | ++ | | k |
| 23 | | 35 | 49 | 8 | 2 | ++ | 4.9 | k |
| 24 | | 35 | 49 | 9 | 7 | - | | k |
| | Average color intensity of Group 3 | | | 8.4 | 5.4 | | | |
| 25 | F.I + 5 mg. folic | 35 | 50 | 8 | 5 | ++ | 3.3 | k |
| 26 | acid and 1 Gm. | 34 | 49 | 10 | 7 | + | | |
| 27 | methylfolic | 34 | 49 | 10 | 7 | ++ | 3.2 | k |
| 28 | acid per kg. | 34 | 49 | 9 | 7 | ++ | 4.1 | k |
| 29 | | 34 | 49 | 10 | 7 | ++ | 7.0 | k |
| 30 | | 34 | 49 | 10 | 7 | + | 2.1 | k |
| 31 | | 34 | 49 | 10 | 7 | | | k |
| 32 | | 33 | 49 | 8 | 5 | ++ | | k |
| | Average color intensity of Group 4 | | | 9.4 | 6.5 | | | k |
| 33 | F.I + 25 mg. folic | 33 | 49 | 9 | 7 | ++ | 3.2 | k |
| 34 | acid and 1 Gm. | 33 | 49 | 6 | 3 | ++ | 2.6 | k |
| 35 | methylfolic | 33 | 49 | 8 | 6 | ++ | 2.9 | k |
| 36 | acid | 33 | 49 | 8 | 6 | ++ | | k |
| 37 | | 33 | 49 | 5 | 1 | ++ | 3.7 | k |
| 38 | | 33 | 49 | 2 | 5 | ++ | 3.6 | k |
| 39 | | 33 | 49 | 8 | 6 | ++ | 2.0 | k |
| 40 | | 33 | 49 | 5 | 2 | ++ | | k |
| | Average color intensity of Group 5 | | | 6.4 | 4.0 | | | |

\+ indicates premature papillary atrophy.
++ indicates the presence of "cysts."
\- signifies the absence of histological specimen.

The pathologic finding most constantly present is a premature, sudden atrophy of the stratum papillare. In Table II (next to last column), the distance from the end of the enamel matrix to the place where the papillary atrophy occurs is indicated. It has not been possible to measure all the preparations, but in all cases where measuring has been successful the distance has been less than the normal 6.0 mm. Fig. 2 shows the appearance of the premature papil-

lary atrophy. In some cases an irregular proliferation, in the incisal direction, of the layer which the papillary atrophy has left behind, takes place (Fig. 3). Following the papillary atrophy there is generally an accentuation of the stratum intermedium, which comes to lie in direct contact with the periodontal connective tissue.

Fig. 1.

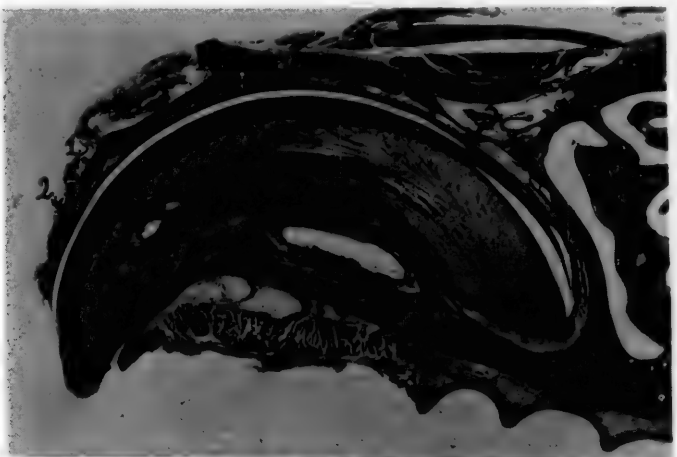

Fig. 2.

Fig. 1.—Low-power magnification of upper incisor of normal, 3-month-old rat. Arrows *1*, *2*, and *3* indicate sites of atrophy of ameloblasts and stratum papillare, and show where the enamel matrix becomes acid-soluble. Optics: tessar 5 cm.

Fig. 2.—Rat No. 12. Sudden termination of stratum papillare. Original magnification: 10 x 12.

In association with, or immediately following the papillary atrophy, edema will develop, apparently originating from the labial connective tissue (Fig. 4). The edema forces its way down between ameloblasts and stratum intermedium, exerting a pressure by which the two layers are separated. In this way a cavity arises which is lined with epithelium on all sides. Fig. 5 shows such a cavity in its initial stage, and Fig. 6 the fully developed cavity lined with epithelium, which bears some resemblance to endothelial cysts. Similar "cysts" may also arise if the edema oozes down between the ameloblasts to be arrested by their

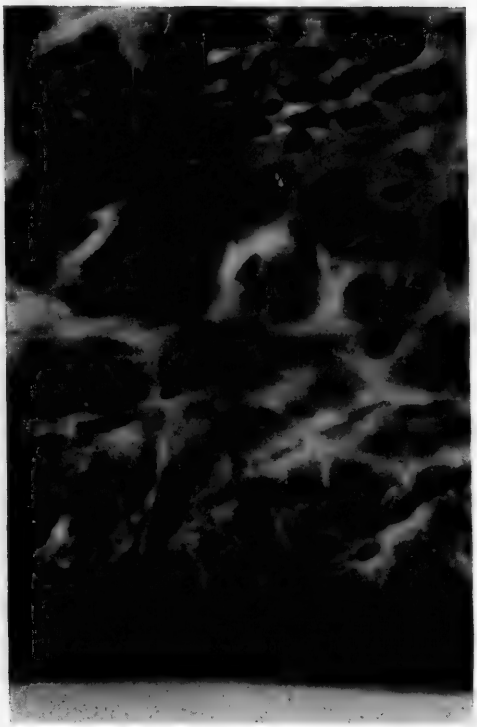

Fig. 3.—Rat No. 10. Intense epithelial proliferation, originating from remnants of the stratum papillare at a level corresponding to crest of facial alveolar bone. Original magnification: 45x6.

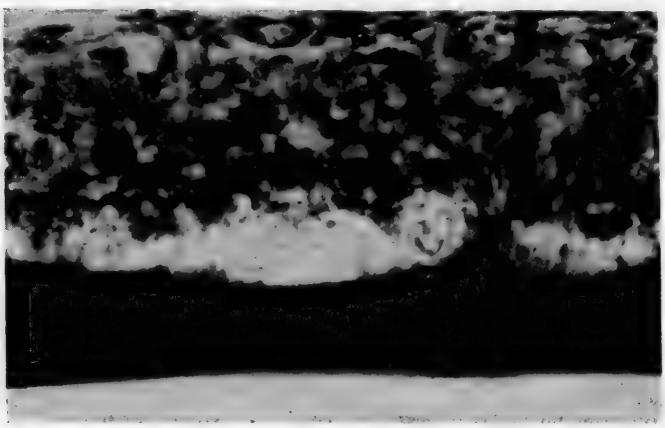

Fig. 4.—Rat No. 27. Development of edema peripheral to ameloblasts. Original magnification: 45x6.

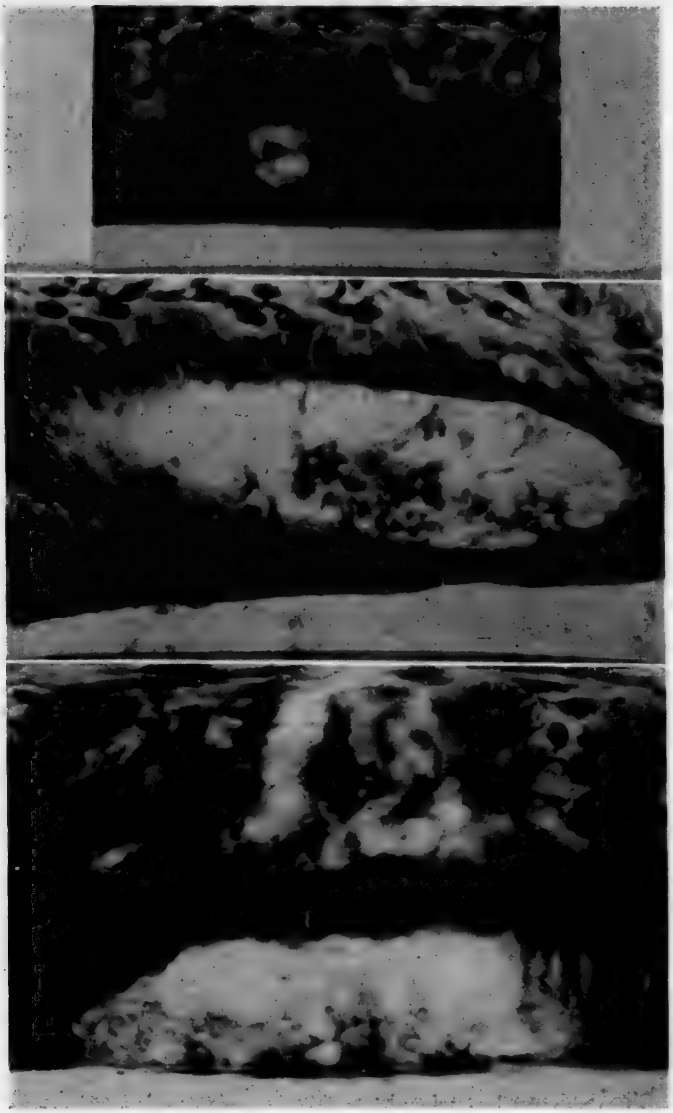

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 5.—Rat No. 28. Initial stage of ameloblastic cyst. Original magnification: 45x6.
Fig. 6.—Rat No. 27. Fully developed ameloblastic cyst. Original magnification: 45x6.
Fig. 7.—Edema located between basal portion of ameloblasts and basal membrane. Original magnification: 45x6.

basal membranes. Gradually as more fluid accumulates, the ameloblasts are pressed toward the periphery (Fig. 7) and a cavity is formed, which is bounded peripherally by ameloblasts, and centrally by the basal membrane of the ameloblasts. Up to eight cysts have been observed in a single enamel organ, the largest of which measured 1.3 mm. in length. Often, the cysts are filled with a stroma, the nature of which cannot be recognized on the basis of the specimens at hand. Table II indicates the frequency with which the cysts occur, + signifying a premature papillary atrophy, and ++ the presence of cysts. There does not appear to be any difference in the frequency of pathologic changes in the five groups.

## DISCUSSION

In 1942, Moore[3] found that whitening of the teeth results from deficiency of vitamin E, and in the same year Irving[4] described "a premature and abnormal degeneration of the enamel organ" in normally pigmented incisors of three rats which had been on a vitamin E deficient diet for 167 days.

Since the diet used in the experiments here reported contained no vitamin E at all, three rats out of a number of rats which had been on the basal diet (F.I) for 66 days and had their incisors depigmented were given 1 mg. of tocopherol daily for 56 days. Table III*A* shows that, except for the lower incisors in one case, the incisors of the animals which received tocopherol had again become pigmented while the control animals had completely depigmented upper incisors. In another experiment rats on the F.I diet were given 3 mg. of tocopherol daily during the whole experimental perriod of from 33 to 70 days. Table III*B* shows conditions identical with those in Table III*A*. Since the typical histopathologic changes also failed to manifest themselves in the animals which had received tocopherol, it would seem justified to conclude that the changes observed, clinical as well as histological, are due to a vitamin E deficiency. A study of Table II shows that histopathologic changes may very well be present in normally pigmented incisors, but that they possibly should be ascribed to the fact that the histologic changes are primary and the depigmentation, secondary.

TABLE III

SIGNIFICANCE OF TOCOPHEROL FOR PIGMENTATION OF INCISORS IN RATS ON VITAMIN E DEFICIENT DIET

| *A* | | | | | *B* | | | | |
|---|---|---|---|---|---|---|---|---|---|
| NO. | DIET | EXPERIMENTAL PERIOD (DAYS) | COLOR OF TEETH | | NO. | DIET | EXPERIMENTAL PERIOD (DAYS) | COLOR OF TEETH | |
| | | | UPPER | LOWER | | | | UPPER | LOWER |
| 116 | Basal diet | 122 | 1 | 6 | 176 | Basal diet | 33 | 10 | 7 |
| 117 | | 122 | 2 | 6 | 177 | | 41 | 10 | 7 |
| 118 | | 122 | 1 | 4 | 178 | | 50 | 4 | 6 |
| | | | | | 179 | | 58 | 3 | 5 |
| 113 | Basal diet + | 122 | 10 | 7 | 180 | | 70 | 2 | 5 |
| 114 | 1 mg. tocopherol daily | 122 | 8 | 6 | | | | | |
| 115 | | 122 | 9 | 1 | 210 | Basal diet + | 33 | 10 | 7 |
| | | | | | 211 | 3 mg. tocopherol daily | 41 | 10 | 7 |
| | | | | | 212 | | 50 | 10 | 7 |
| | | | | | 213 | | 58 | 10 | 7 |
| | | | | | 209 | | 70 | 10 | 7 |

The results here reported are not in accordance with the findings of Dam and Granados,[5] "that depigmentation of incisors in vitamin E-deficient rats requires the presence of fat, presumably unsaturated fatty acids, in the diet." There must be some other factors, unknown so far, which are responsible for the depigmentation since the diet used in the experiments here reported had only a fat content of 3 per cent.

Among the previously reported symptoms of vitamin E deficiency, mention should also be made of Pappenheimer's[16] finding of interstitial fibrinous edema in the muscular tissue. This result is in accord with the edemas which these studies have demonstrated to be present in the enamel organ.

The data presented in Table II and the results subsequently obtained (Tables III*A* and *B*) would seem to warrant the conclusion that the changes observed do not bear any relation to the content of folic acid or methylfolic acid in the diet, but should probably be attributed to a deficiency of vitamin E. The methylfolic acid, on the other hand, has a markedly destructive effect on the periodontal tissues of the rat molars. This destructive effect will be dealt with in some detail elsewhere.[7]

## SUMMARY

On a purified diet containing 0.1 per cent of succinylsulfathiazole and deficient in vitamin E, a depigmentation of the incisors and a series of histopathologic changes have been ascertained in 39 white rats. There was premature atrophy of the stratum papillare, marked edemas, and cyst formations. Rats treated with tocopherol did not exhibit these changes. The conclusion is drawn that the changes are attributable to a vitamin E deficiency.

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# THE EFFECT OF X-RAY IRRADIATION ON THE TEETH AND SUPPORTING STRUCTURES OF THE MOUSE

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THIS study was undertaken to observe the response of the developing teeth and supporting structures of young mice to x-radiation. A survey of the literature revealed a scarcity of experimental data on this subject. Moreover, much of the previous work would be difficult to repeat because of a lack of standardization of the older units of x-ray dosages.

Numerous investigators have observed and reported the effect of x-ray irradiation upon the growth and development of bones. This material is well summarized in the reviews by Colwell,[1] Duggar,[2] Gates,[3] and Desjardins.[4] Hemiatrophy and osteonecrosis as well as morphological abnormalities and exfoliation of the dentition have resulted from radiation therapy.[5-8]

One of the earliest recorded experiments dealing with the effect of x-rays upon the teeth and jaws was reported by Tribondeau and Recamier[9] in 1905. These workers irradiated one side of the face of a young cat and observed that there was a retardation in the development of the dentition on the irradiated side. No histologic studies were made.

Leist[10] exposed 8 rats to dosages varying from 1.5 to 15 Holzknecht units (1 Holzknecht or H equals approximately 1/5 S.E.D. or skin erythema dose). Histologically, the odontoblasts were disorganized and there was an abnormal formation of dentinoid material within which odontoblasts were trapped. The ameloblasts, enamel, and cementum were not affected. In two young dogs, 32 to 60 Holzknecht units produced similar changes. Atrophy of the pulp resulted, and the eruption of the teeth was retarded. Leist concluded that the odontoblasts are more radiosensitive than the ameloblasts.

The work of Smith[11] is similar to that of Leist. Smith states that these changes are temporary and that there is a recovery of growth potential of the injured tooth.

More recently, Dale[12] studied the incisor teeth of rats which received total body irradiation. Thirty weanling and thirty adult rats were irradiated with exposures of 1, 375, 500, 625, and 750 roentgen units. Macroscopic examination revealed edematous and bleeding gingivae and enamel hypoplasia. Histologically, there was a retardation or cessation of amelogenesis and odontogenesis. Irregular, discolored enamel rods with inclusion bodies in the interprismatic substance were observed. Dale stated that the enamel is more radiosensitive than the dentin.

This material is taken from a thesis submitted to the faculty of the Graduate School of Washington University in partial fulfillment of the requirement for the degree of Master of Arts in Pathology.

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## METHODS

Forty-four Swiss and $C_{57}$ black mice, 2 to 28 days of age, were irradiated in the area of the mandibular joint and the molar teeth. Littermates and littermate controls were used whenever feasible. All animals were maintained on Purina Laboratory Chow, with water available at all times.

A Philips Metalix contact x-ray machine was used under the following constant conditions: k.v. 50, Ma. 2, t.s.d. 20 mm., 0 Filter, H.V.L. 0.3 mm.Al (approximately 5,000 r per minute). The quality of radiation is relatively soft, and the intensity of radiation diminishes greatly with the depth of the tissues. Thus, at a depth of 4 mm. in soft tissue, the dosage is approximately half of that at the skin surface. The diameter of the field to be irradiated was regulated by the use of cones or ports, the openings of which varied from 5 to 14 mm. in diameter. The three dosages which were used were 1,500 r (18½ seconds), 3,000 r (38 seconds), and 5,000 r (62 seconds).

Fig. 1.—Occlusal imbalance of incisors in 5-week-old mouse, four and one-half weeks following irradiation.

Following irradiation, thirty-two of the mice which survived were sacrificed at time intervals from five to thirty-seven days. Littermate controls were sacrificed to coincide with these time intervals. The weights and sex of both irradiated and control animals were recorded. The heads of mice under 3 weeks of age were fixed and decalcified in Bouin's solution (with 10 per cent acetic acid). The heads of older mice were split longitudinally and fixed in 10 per cent formalin, after which each half of the head was radiographed. All heads were decalcified, embedded in paraffin, serially sectioned in either a frontal or sagittal plane, and stained with hematoxylin and eosin.

## RESULTS

*Gross observations.*—Erythema and subsequent epilation of the irradiated skin surface occurred four to six days following irradiation. With 3,000 to 5,000 r there was a necrosis of the skin and underlying tissues. Administration of 1,500 r to animals under 4 days of age caused a hemiatrophy of the mandible which was noticeable after two weeks (Fig. 1). This change was not as prominent with 3,000 and 5,000 r. There was a retardation of the rate of eruption of the incisor tooth on the irradiated side. Roentgenologically, there was an absence of development of root structure on the side of the animal which received the heaviest irradiation (Fig. 2). Those animals which were heavily irradiated (5,000 r) over a small area at the apex of the incisor tooth exhibited developmental abnormalities such as spiked, malformed, or hypoplastic incisors, or failure of the incisors to erupt. Dosages of 3,000 to 5,000 r produced a generalized retardation of development in the experimental animals as compared with littermate controls.

*A.*

*B.*

Fig. 2.—Radiograph of left and right side of mouse head. *A* (irradiated side) showing lack of development of third molars and absence of molar root structure.

*Microscopic observations.*—Histologic examination of the teeth and jaws of animals exposed to 5,000 r revealed marked disturbances in dentinogenesis, amelogenesis, and osteogenesis. These changes were more prominent on the irradiated side.

Seven days following irradiation of 2-day-old mice the structure of the third molar teeth is distorted (Figs. 3, 4, and 5). No normal ameloblasts are

visible. The cells along the inner enamel epithelium vary from low cuboidal to squamous in type, and there is little or no evidence of enamel matrix formation. The odontoblastic layer is not intact and the remaining odontoblasts appear as large, irregular, polyhedral cells with cytoplasmic processes. Many of these cells are trapped within irregular areas of calcified material (osteodentin) in which there are no visible dentinal tubules (Figs. 4 and 5). Osteodentin may be formed throughout the pulp chamber. The growing end of the incisor tooth exhibits similar changes in the irradiated area. The ameloblastic layer of the other molar teeth on the irradiated side appears to be intact in certain areas, while in other areas, especially toward the cervical part of the crown, the ameloblasts appear as cuboidal or squamous cells. Enamel matrix is present only where the ameloblasts are intact. Some ameloblasts exhibit degenerative changes.

The oral epithelium is desquamated in the area of the mucobuccal fold and there is a subepithelial infiltration of heterophil leucocytes. The epithelium is replaced by strands of keratohyalin material and the adjacent oral cavity contains basophilic staining debris and leucocytes (Fig. 6).

There is diminution in the cellularity of the bone marrow of the jaws with increased vascularity. The number of osteoblasts is decreased and there is less trabecular bone formation as compared with normal controls.

Changes nine and ten days after irradiation of 2-day-old animals are similar, except they are of greater severity. In several mice the degenerating ameloblasts appear as mononuclear ovoid cells which resemble macrophages. The alveolar bone of the jaws is thin and there is an aplasia and early fibrosis of the marrow.

Those animals which received 3,000 r at 2 days of age exhibited the following changes five days postirradiation. Osteodentin formation with trapped abnormal odontoblasts is present in the pulp. In some teeth there is an irregularity of enamel matrix and dentin, which gives portions of the crown of the tooth a scalloped appearance. The ameloblasts appear as low cuboidal cells in the third molars on the irradiated side, and are present only on the occlusal surface of the tooth. The ameloblasts of the other molar teeth are normal in appearance. The maxilla and mandible show lack of development, with retardation of bone formation, and there is desquamation of the oral epithelium on the irradiated side.

The teeth of 3-day-old mice which received 1,500 r exhibit some formation of osteodentin with abnormal odontoblasts (seven days following irradiation). The ameloblasts are not visibly changed, and the enamel matrix appears normal. Bone formation and marrow cellularity are normal.

Twelve days following irradiation (5,000 r of 5- to 7-day-old animals) the structure of the third molar teeth is similar to what has been described. However, the third molars in some mice are more severely distorted (Fig. 7). In the enamel organ of the first and second molars, the ameloblasts are low cuboidal to squamous in type. Between the inner and outer enamel epithelium are masses of keratohyalin material, groups of large polyhedral cells, and degenerating leucocytes.

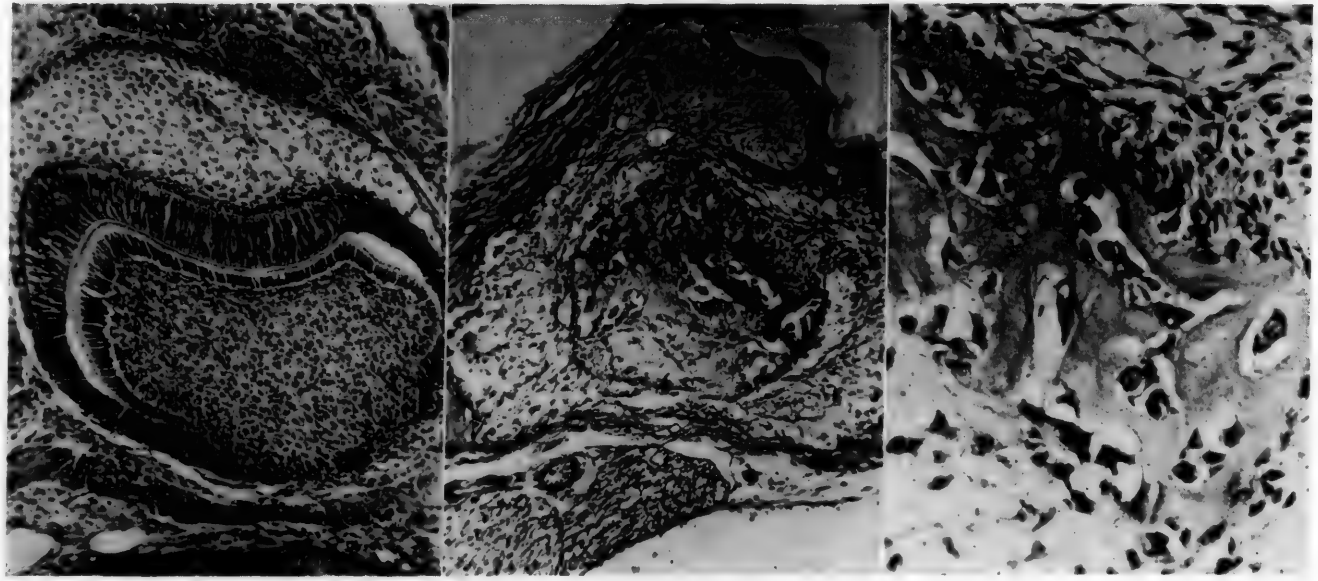


Fig. 3. Fig. 4. Fig. 5.

Fig. 3.—Third molar tooth of normal 9-day-old mouse.
Fig. 4.—Third molar tooth of 9 day-old mouse, seven days following irradiation.
Fig. 5.—High power of Fig. 4, showing abnormal odontoblasts within osteodentin.

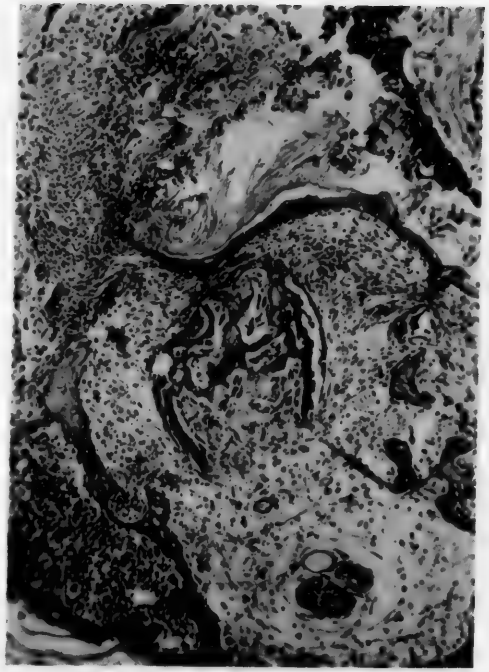


Fig. 6.

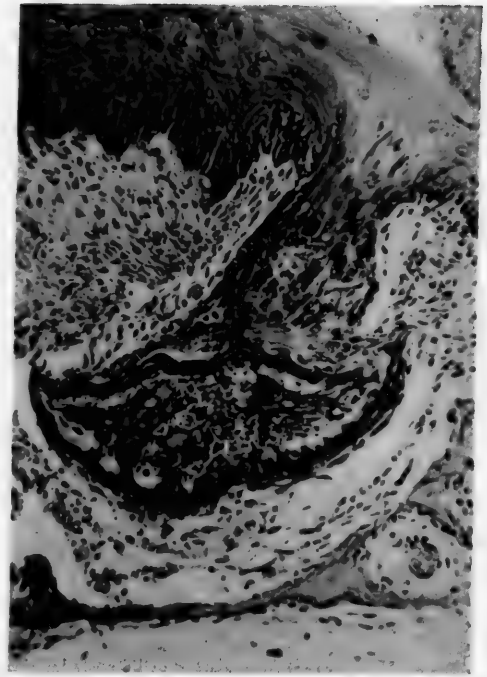


Fig. 7.

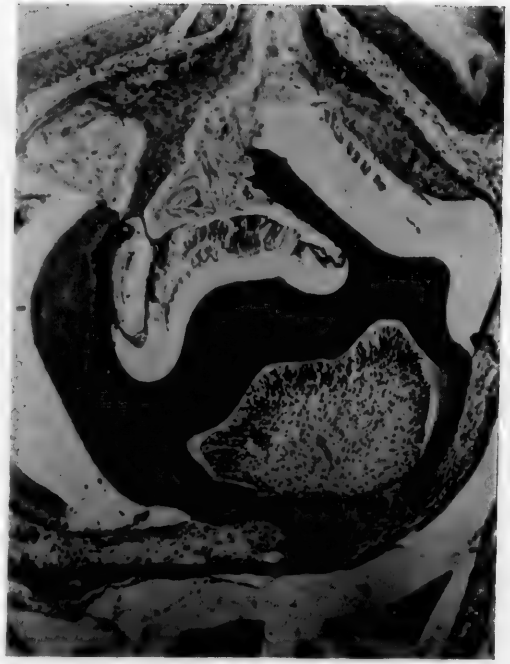
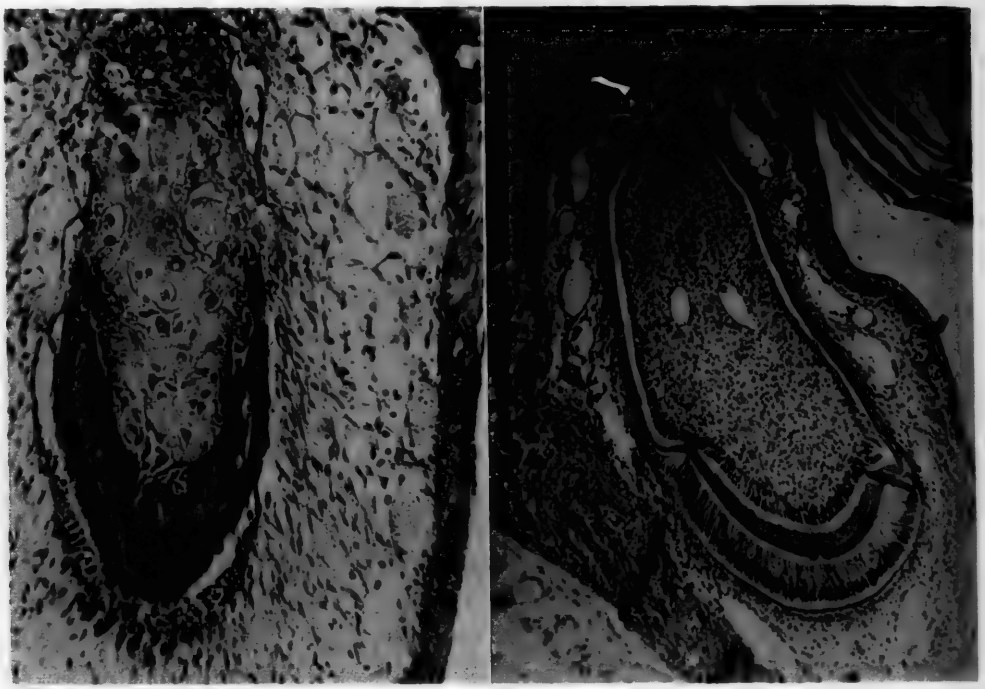


Fig. 8.

Fig. 6.—Desquamation of oral epithelium seven days following irradiation. Distorted third molar is also present.
Fig. 7.—Severely distorted third molar of 19-day-old mouse, twelve days following irradiation.
Fig. 8.—Second molar of 19-day-old mouse, twelve days following irradiation. Note the abnormal apical root structure.

The root structure of the first and second molar teeth was not developed normally. The apical portion of the root consists of a mass of dentinoid material within which are globular masses of a lightly basophilic substance. This dentinoid-globular material contains a few trapped cells and is often fused to the underlying alveolar bone (Fig. 8). The coronal dentin is well developed and the pulp appears normal.

The changes in the growing end of the incisor teeth are identical with those seven days postirradiation (Fig. 9). There is a marrow aplasia and fibrosis with a predominance of heterophil leucocytes in the marrow spaces in the maxilla and mandible.



*A.* *B.*

Fig. 9.—*A*, Growing end of incisor of 19-day-old mouse, twelve days following irradiation. *B*, Growing end of incisor of normal 19-day-old mouse.

The desquamation of the oral epithelium is not as severe as that which occurred when 2-day-old mice were irradiated.

Eighteen days after exposure to 5,000 r (4-day-old mice) the first molar teeth are the only teeth which have erupted. The changes are similar to those described at twelve days postirradiation. The growing end of the incisors on the irradiated side still exhibit abnormalities of amelogenesis and odontogenesis.

The changes twenty days after exposures of 1,500 and 3,000 r are similar to the earlier changes except that failure of root development is prominent. However, the development of the root structure is less adversely affected than with 5,000 r (Fig. 10).

At twenty to thirty days postirradiation the inflammatory response within the tooth follicle and soft tissues is prominent. The enamel organ of the un-

Fig. 10.—First molar tooth of 27-day-old mouse, twenty days following irradiation. Note the absence of well-developed root structure.

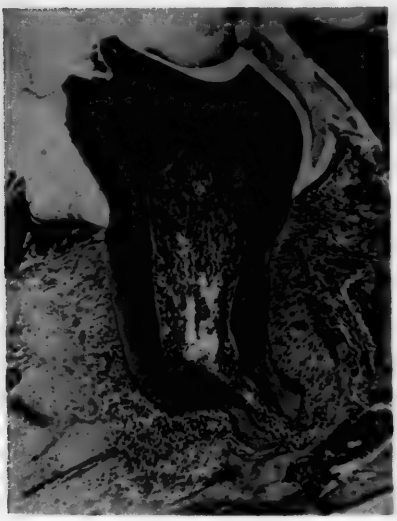


Fig. 11.

Fig. 12.

Fig. 11.—Third molar of normal 27-day-old mouse.

Fig. 12.—Third molar of 27-day-old mouse, twenty days following irradiation (high magnification).

erupted molars contains large numbers of large polyhedral cells with pale staining cytoplasm and numerous degenerating leucocytes. The interdental papilla and the periodontal membrane are infiltrated with inflammatory cells. There

are numerous microabscesses, some of which coalesce in the adjacent soft tissue. The structure of the third molar teeth varies considerably, but all are markedly altered. The tooth germ lies deep in the investing tissues. Some are irregular masses of dentinoid material, while others are more normal in structure (Figs. 11 and 12).

The basal bone is poorly formed and exhibits prominent cement lines. There is a marrow aplasia and fibrosis. The alveolar process of the maxilla may be absent. Heterophil leucocytes represent the predominating marrow cell type. Osteoblasts are more numerous than in earlier postirradiation stages, but their number is not as great as in the unirradiated controls.

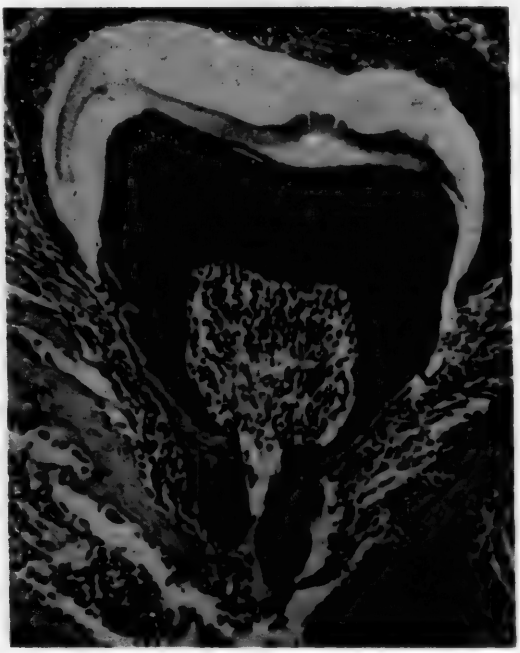


Fig. 13.—Third molar of 43-day-old mouse, thirty-seven days following irradiation (high magnification).

Thirty-seven days following the irradiation (5,000 r) of a 6-day-old mouse, the oral epithelium in the third molar area is one or two cells thick; in some areas there is no continuity of the epithelium. The lamina propria is densely infiltrated with heterophil leucocytes and lymphocytes. The adjacent oral cavity contains fragments of hair, leucocytes, and cellular debris. Multiple abscesses are present in the soft tissues adjacent to the maxilla. The first and second molars have partially erupted. The third molars are unerupted and are surrounded by squamous type cells which represent the reduced enamel organ (Fig. 13).

Sixty days following the irradiation of 1-week-old mice, the pulps of some of the molar teeth are atrophied and contain a lightly basophilic fibrinous

network within which are scattered pyknotic nuclei (Fig. 14). The pulps of other molar teeth exhibit a marked fibrosis. The marrow is fibrotic in some areas but in other areas the marrow cellularity appears normal.

Mice (28 days old) which were irradiated with 5,000 r, nine days after exposure, exhibited a desquamation of the oral epithelium with a replacement by a keratohyalin material which is infiltrated with leucocytes. The mass of desquamated epithelium and debris also contains clumps of leucocytes. The interdental papillae are necrotic and in some areas have been obliterated.

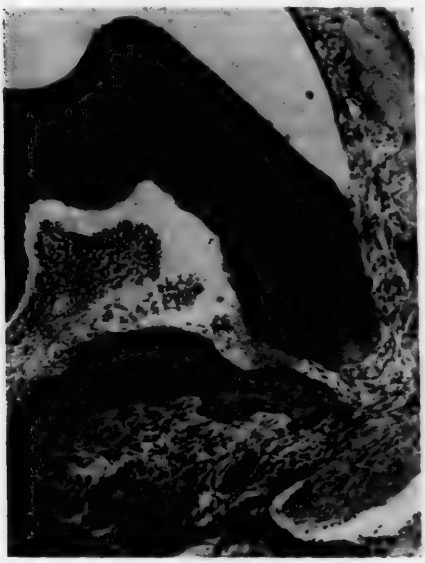


Fig. 14.

*A.*

Fig. 15.

Fig. 14.—Second molar tooth of 67-day-old mouse sixty days following irradiation. The pulp is atrophic and the root structure is rudimentary.

Fig. 15.—Roots of first and second molars of 6-month-old mouse five months following irradiation. Note the ankylosis of the root structure to the alveolar bone, *A*.

The marrow cellularity of the body of the mandible is reduced, and there is a preponderance of leucocytes as compared with the bone marrow of normal controls. The periodontal membrane is necrotic in some areas and its capillaries are dilated.

There are no changes in the molar teeth. However, the growing ends of the incisor teeth exhibit ameloblastic destruction, osteodentin formation, and increased vascularity of the pulp. The ameloblasts are normal or exhibit slight degenerative changes, while the odontoblasts are distorted and appear as polyhedral cells.

Three months following exposure (5,000 r) of 28-day-old animals there is an ankylosis of the roots of the molar teeth to the supporting alveolar bone which is poorly formed and exhibits prominent cement lines. The periodontal membrane is aplastic. The pulps of some teeth are atrophied while the pulps of others are fibrotic or filled in by osteodentin. There is abscess formation

in the adjacent tissues. Six months following the same dosage, the ankylosis is very pronounced (Fig. 15), and there is still evidence of an inflammatory response in the adjacent soft tissues. The marrow cellularity appears normal except for certain areas which are fibrotic. The endosteum appears to have been destroyed adjacent to these areas.

## DISCUSSION AND SUMMARY

The advantages in using a contact type x-ray machine are: first, the intensity of radiation per unit time is high, and second, the intensity of radiation falls off rapidly at a depth of several millimeters. The disadvantage in using a contact machine is that a very close apposition between the port and the tissue to be irradiated is necessary in order to determine accurately the dosage. This close apposition is sometimes difficult to establish. The recorded dosages represent the intensity of radiation at the skin surface, and do not represent the intensity at the underlying tooth and bone structure. These factors may help to explain the lack of uniformity of some of the histologic findings.

It is apparent that the degree of damage to the teeth and jaws is dependent upon the age at which the mouse is irradiated. The degree of damage to the tooth structure is also dependent upon the stage of histogenesis of the individual tooth. Thus, the third molar tooth germs of animals which are irradiated at 2 days of age exhibit more destructive changes than the other molar teeth.

The molar teeth of 28-day-old mice are unaffected by 5,000 r. However, the growing incisor teeth exhibit marked destructive changes one week following irradiation.

Following the irradiation of animals under 7 days of age the ameloblasts of the third molars, if still present, will be found adjacent to the areas of the crown which were formed prior to exposure. There is less ameloblastic destruction in the area of the future cusps where the ameloblasts have first differentiated. Also, enamel matrix is present only in these areas. This suggests that there is a disruption or complete cessation of ameloblastic histogenesis. The resistance of the older ameloblasts indicates the relative radiosensitivity of the young ameloblasts. The presence of squamous and low cuboidal cell types in the ameloblastic area suggests a morphological change of cell type or a replacement metaplasia.

Both young and mature odontoblasts are more radiosensitive than ameloblasts. With dosages of 1,500 and 3,000 r the odontoblasts are distorted while the ameloblasts and enamel matrix are relatively intact. The altered odontoblasts have some function since an irregular, amorphous, eosinophilic matrix is formed.

Two to three weeks following irradiation of animals under 1 week of age, the coronal dentin of the first and second molars appears relatively normal. However, the developing root structure has been markedly affected. The odontoblasts are most distorted at the apical portion of the tooth. Again, this indicates that the more undifferentiated odonoblasts exhibit a greater radiosensitivity.

Irradiation may alter the process of root formation in several ways. First, the mesenchymal pulp may be damaged so that histodifferentiation of odontoblasts cannot occur. Second, irradiation may damage the sheath of Hertwig.

## CONCLUSIONS

The degree of radiation damage to the teeth and jaws of mice is dependent upon the age at which the animal is irradiated and the stage of histogenesis of the individual tooth.

With dosages of 5,000 r the cells in the ameloblastic layer appear as low cuboidal or squamous type cells. There is a cessation of histodifferentiation of new ameloblasts. Dosages of 1,500 to 3,000 r produce less marked changes.

Dosages of 1,500 to 5,000 r severely damage the odontoblastic layer. The surviving odontoblasts resemble osteoblasts and an irregular, amorphous, acidophilic staining substance (osteodentin) is formed.

There is a retardation or cessation of root formation (first and second molars) following exposures of 1,500 to 5,000 r, while the development of the coronal dentin is only slightly altered.

Odontoblasts are more radiosensitive than ameloblasts.

The development of the basal and alveolar portions of the jaw is retarded or stopped completely.

There is an acute inflammatory response within the enamel organ, and in the adjacent soft tissues.

The late postirradiation changes include atrophy and fibrosis of the pulp and ankylosis of the root to the alveolar bone.

The author wishes to express his appreciation to Dr. B. M. Levy, Columbia University School of Dental and Oral Surgery, for invaluable assistance in this project, and to Mrs. Mary Gross and Miss Dorothy Krug for technical assistance.

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# CHANGES IN THE KALLIKREIN CONTENTS OF SALIVA UNDER PHYSIOLOGICAL CIRCUMSTANCES AND IN PERIODONTOCLASIA

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KALLIKREIN (Padutin by proprietary name) was discovered by Frey and Kraut.[1] It is a hormonal substance, produced largely in the pancreas and has a vasodilating effect. One part of it reaches the blood circulation directly, and the other finds its way to the duodenum through the pancreatic duct. Kallikrein is constantly present in the blood in an inactive form and is excreted in the urine in active form.

The effect of kallikrein is observed, mainly, on the walls of the capillary vessels. Where there is a stagnation in the tissues and as a result the pH of the blood approaches acidity, kallikrein is liberated from its inactive form, changes into the active, and increases the permeability of the capillary walls. This explains the sudden drop of the blood pressure of experimental animals following intravenous administration of kallikrein. The blood pressure, however, returns to the original level soon, because kallikrein is inactivated by the blood.[2, 3]

Besides the pancreas, kallikrein is produced in the salivary glands, mainly in the submandibular gland and in lesser degree in the parotid and the sublingual glands. These glands produce active kallikrein simultaneously with saliva, the latter containing about two kallikrein units in one cubic centimeter. One kallikrein unit is generally equal to the amount of kallikrein in 5 c.c. of human urine.

An experimental animal with its pancreas extirpated still excretes kallikrein in its urine, although in a much smaller concentration.[5] After the administration of insulin in such animals, the kallikrein excretion increases, the Kallikrein here being produced in the salivary glands and finding its way to the blood circulation, finally to be excreted in the urine. These experiments proved that kallikrein is produced by both the pancreas and the salivary glands independently. The kallikrein of the saliva reaches the stomach and is destroyed there by the hydrochloric acid of the gastric juice. Thus, the oral cavity is the only place in the organism where kallikrein is constantly present in its active form.

It seems probable that kallikrein produces some effect on its environment, both in the intestinal tract and in the oral cavity. Very likely the kallikrein contents of the saliva is not indifferent to the oral mucous membrane which is constantly moistened by the former. The capillary vessels of the mucous membrane are situated superficially so that substances from the oral cavity are easily absorbed and get in the blood circulation.

## EXPERIMENTAL

On the basis of these considerations, experiments were started to reveal whether there are any changes in the kallikrein contents of the saliva in cases of periodontal disease where local pathogenic factors can be omitted and the disease may be attributed to some unknown systemic change (periodontosis).

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For the sake of gaining a correct estimate in the above cases, the changes of the kallikrein contents of the saliva under physiological conditions had to be determined as a control.

*Method*

Conclusions on the kallikrein content of the saliva were drawn from the changes of the blood pressure of atropinized dogs. To achieve narcosis 0.4 c.c. of Pernoston (10 per cent solution of sodium butyl $\beta$ bromallylmalonylurea) plus 0.01 Gm. of morphia and 0.005 Gm. of atropine per kilogram was administered to the experimental animal. The atropine was given for the elimination of the eventual acetylcholine effect. Artificial respiration was used and the blood pressure was registered by a manometer inserted in the carotid.

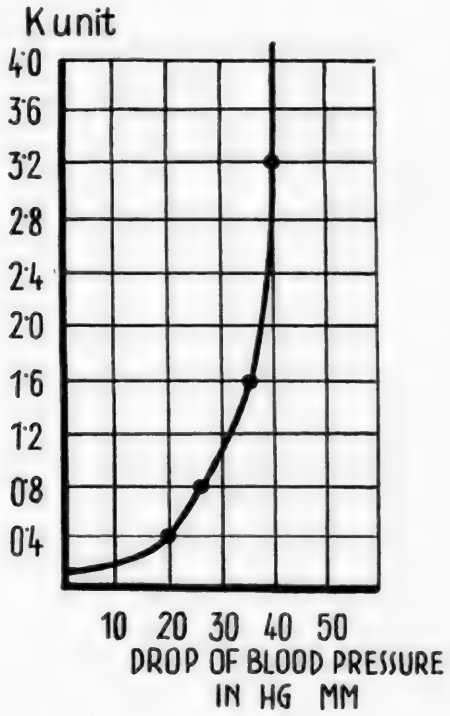


Fig. 1.

The necessary amount of saliva was taken from the patients after two hours of starvation. The saliva, produced spontaneously, was collected by a saliva ejector, precipitated, centrifuged for a short time, and injected on the same day. One c.c. of saliva, treated in the above way, was injected in the femoral vein of the dog. Since there is a tachyphylaxis toward kallikrein, the injections were given at 30-minute intervals and not more than 4 to 5 injections were administered to the same experimental animal. For the sake of comparison, saliva of old and young, diseased and healthy individuals was given alter-

nately to the same animal. In the evaluation of the experiments, a standard kallikrein preparation (Padutin) was used. From the data of the increased dosage of kallikrein on one hand and from those of the drop of the blood pressure in millimeter of mercury on the other, a curve was constructed which is shown in Fig. 1. Every experimental animal received Padutin as a control. The results were arrived at through comparing the data of the saliva injections with the Padutin curve.

*Examinations of the kallikrein contents of saliva under physiological circumstances.*—The kallikrein contents of the saliva of 14 young (8 to 18 years) and 14 older (45 to 78 years) individuals (both male and female) were determined. The teeth of the young were absolutely healthy, those of the middle-aged were mostly free of recent caries and periodontal disease. The patients of advanced age were edentulous. Whether the secretion of the saliva was slow or fast was also noted. In many cases the examinations were made on different days, at different times of the day, but always following two hours of starvation.

After reducing the secretion of saliva by administration of atropine, the kallikrein contents has not shown appreciable differences. Neither were any regular changes to be observed in saliva secreted slowly or fast, sparsely or copiously. Only age seemed to be a physiological factor which influenced the kallikrein contents of saliva (Table I). One c.c. of saliva of the patients between 8 to 18 years of age contained 0.3 to 1.8 units of kallikrein (the latter, however, only in a single case). Saliva of the age group 45 to 78 years contained 2 to 8 units (one individual had only 1.1 unit).

TABLE I

| NO. | AGE GROUP 8 TO 18 YEARS | | AGE GROUP 48 TO 78 YEARS | |
|---|---|---|---|---|
| | AGE | K UNIT | AGE | K UNIT |
| 1 | 8 | 1.8 | 45 | 4.8 |
| 2 | 9 | 1.0 | 50 | 3.0 |
| 3 | 11 | 0.3 | 55 | 8.0 |
| 4 | 11 | 0.8 | 57 | 2.0 |
| 5 | 11 | 1.4 | 57 | 4.0 |
| 6 | 12 | 0.4 | 57 | 8.0 |
| 7 | 12 | 1.6 | 60 | 1.1 |
| 8 | 13 | 0.7 | 60 | 5.0 |
| 9 | 13 | 1.0 | 60 | 5.0 |
| 10 | 13 | 4.8 | 62 | 2.3 |
| 11 | 18 | 1.0 | 62 | 6.5 |
| 12 | 18 | 1.2 | 70 | 8.0 |
| 13 | 18 | 1.3 | 76 | 6.5 |
| 14 | 18 | 1.4 | 78 | 6.4 |
| | $M_1 = 1.33$ | | $M_2 = 5.04$ | |
| | $\sigma M_1 = 0.085$ | | $\sigma M_2 = 0.62$ | |

The average value ($M_1$) in the first group was: $M_1 = 1.33$

The average value ($M_2$) in the second group was: $M_2 = 5.04$

$\sigma M_1 = 0.085$ $\sigma M_2 = 0.62$. From this the significant difference is $S = 5.53$. This means that the above difference really exists.

*Examinations of the kallikrein contents of saliva in periodontal disease.*—The kallikrein contents of the saliva of patients suffering from periodontal disease was determined. During the course of these investigations, cases in

which local pathogenic factors, such as occlusal trauma, ulcerative gingivitis, "Schmutzphorrhoë" or some systemic diseases like scurvy and diabetes, could be made responsible for the development of periodontal disease were eliminated.

The kallikrein content of saliva was investigated in 6 cases of periodontal disease (primary parodontoclasia). Identical investigations were carried out as a control in 5 patients of the same age, whose teeth and gingiva were perfectly healthy. The examinations were extended to 6 more cases, 3 of which had ulcerative gingivitis, not treated as yet, and the remaining 3 having shown a marked predisposition to dental caries. All of these 17 patients were otherwise perfectly healthy.

Neither in the caries cases nor in those with ulcerative gingivitis was a difference in comparison with the control cases found. In the periodontal disease cases, however, the values were considerably higher. The kallikrein content of the saliva in these cases was generally 5 to 6 times greater than that in the control patients of the same age. The absolute values found in the periodontal disease cases were in direct proportion to the age of the patient. On the other hand, the younger the patient was, the larger was the relative kallikrein content in comparison to that of the control cases. This seems to be explained by the fact that the prognosis of periodontal disease developing in younger individuals is definitely more unfavorable than in more advanced age.

TABLE II

| NO. OF EXPERIMENTAL ANIMAL | PERIODONTAL DISEASE CASES | | CONTROL CASES | | |
|---|---|---|---|---|---|
| | AGE | NUMBER OF K UNITS IN 1 C.C. OF SALIVA | AGE | COMMENTS | NUMBER OF K UNITS IN 1 C.C. OF SALIVA |
| X | 30 | 6.5 | 28 | Healthy teeth | 1.2 |
| | | | 28 | Carious teeth | 1.6 |
| | | | 37 | Ulcerous gingivitis | 1.3 |
| | | | 46 | Ulcerous gingivitis | 1.2 |
| XI | 43 | 6.5 | 43 | Healthy teeth | 3.1 |
| | 45 | 7.0 | 54 | Healthy teeth | 0.4 |
| XII | 46 | 5.0 | 43 | Carious teeth | 1.4 |
| | | | 42 | Ulcerous gingivitis | 0.8 |
| XIII | 28 | 6.0 | 28 | Healthy teeth | 1.1 |
| | 62 | 12.0 | 60 | Healthy teeth | 5.0 |
| | | | 39 | Carious teeth | 7.0 |

Atrophic processes of the gingiva developing in more advanced age bear some resemblance to the physiological aging of the periodontium. Care was taken to select younger persons for the investigations. The authors were successful in their endeavor with one exception which seems worth mentioning. This individual was a 62-year-old male patient on whom, far beyond the usual regressive changes of his age, a very severe degree of diffuse atrophy involving all his many remaining teeth was observed. The kallikrein content of his saliva was the double of the usual value of 5 to 6 units corresponding with that age, which seems to indicate pathological increase of kallikrein. Two younger patients (28 and 30 years of age), possessing most of their teeth, had shown symptoms of periodontosis and lost a few teeth on account of atrophy. Three of

the patients, of 43, 45, and 46 years of age, had atrophy of II and III degree; their gingivae were free of inflammatory symptoms but were livid and there were deep pockets and moderate loosening of the teeth.

Though one could hardly draw final conclusions from such a small quantity of material, it seems to be a useful basis for further investigations. Still, the comparison of the physiological and pathological results may support the theory arrived at through these investigations, that in periodontal disease there is a premature aging of the periodontium. The aging of the organism is not a uniform process, neither in time nor in degree, but the increase of kallikrein may be considered as a biological process of diagnostic significance, indicating aging. The premature occurrence of this increase is largely influenced by constitutional factors. In dogs with their pancreas extirpated, the kallikrein supply is produced by the salivary glands and may be increased by administration of insulin. It is probable that the salivary glands are also subjected to the regulating effects of the hormonal correlation of the organism.

The first stage in every inflammatory process is the increase of the permeability of the capillary walls[8] which occurs, according to Frey, when the pH of the blood approaches acidity and kallikrein is liberated from its inactive form. This local effect may be considered in our cases. It is probable that kallikrein has no effect whatsoever on the normal mucous membrane of the oral cavity as it is inefficacious on the mucosa of the intestinal tract. If the continuity of the gingival epithelium suffers injuries, a point of aggression presents itself for the kallikrein. The effect of kallikrein increasing the permeability of the capillaries may result in stagnation and inflammation on the gingival margin, which of course is disadvantageous for the tissues of the periodontium.

## SUMMARY

1. The kallikrein content of the saliva is about 4 to 5 times greater with old age than younger age.

2. In periodontal disease provoked by constitutional factors (periodontosis), the kallikrein content of the saliva is 5 to 6 times greater than in healthy control patients of the same age. There are no considerable differences in caries and ulcerative gingivitis. The amount of the kallikrein is in direct proportion to the progress of periodontosis.

3. It may be presumed that the increase of kallikrein in periodontal disease is provoked prematurely by the same changes as under physiological circumstances in old age. The increased kallikrein content of saliva may damage the periodontal tissues locally.

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# COMPARATIVE STUDIES OF STREPTOMYCIN AND PENICILLIN IN THE TREATMENT OF INFECTIONS OF THE ORAL MUCOSA

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ACUTE infections of the oral mucosa are treated first by medication to control the bacterial invasion, followed by removal of the predisposing factors to prevent recurrence and to raise tissue resistance. Topical application gives a higher concentration at the site of the infection than does parenteral administration and is, therefore, the method of choice.[1, 2]

Since the average dental office is not equipped for bacteriologic examinations, the medicament should be effective against the entire oral flora. It should be rapid in action so that instrumentation may begin as early as possible, and have no deleterious effect upon the hard and soft tissues. The value of penicillin for treatment of such infections has been established.[3] The purpose of the present study is a comparison of penicillin and streptomycin in vitro, in laboratory animals and in clinical use.

## EXPERIMENTAL

### *In Vitro*

In order to duplicate clinical conditions as closely as possible, the motility test was employed using saliva from an acute Vincent's infection. This method has been described elsewhere.[4, 5]

Under the dark-field microscope, a solution of penicillin G, 20,000 units per c.c. of distilled water, instantly stopped the motility of spirochetes and vibrios. Motile bacilli retained their motion for 2 minutes. As previously described,[4, 5] equal parts of the penicillin solution and the saliva were mixed and allowed to stand for 15 minutes when 2 loopsful were introduced into nutrient broth, agar, and brewers' broth. There was no growth in 72 hours. Since two instances of gingival irritation followed clinical application of this penicillin solution, it was given no further consideration. A concentration of 10,000 units per c.c. was also tested. There was no growth in the culture media. However, the time for loss of motility was extremely variable. The solution had limited power of penetration through the solids included in the specimen. If the saliva contained little debris, the motility of most organisms stopped on contact. When the content of solids was high, the solution failed to reach many of the organisms, and their movement continued indefinitely. Thus, the time required for cessation of motion varied with the amount of debris present.

Streptomycin was similarly examined, using 100 mg. per c.c. of distilled water. Motility of all organisms stopped immediately and penetration of debris was rapid. There was no growth in the culture media.

The penicillin and streptomycin solutions were allowed to stand in closed containers exposed to daylight at room temperature; the areas of inhibition were

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measured on streaked plates. Penicillin retained a satisfactory efficiency for 24 hours, streptomycin for 7 days.

*Laboratory Animals*

Two guinea pigs of approximately the same weight were used. One-tenth c.c. of saliva was injected subcutaneously in the right inguinal region of each. In the left thigh, 0.1 c.c. of physiologic saline solution was similarly introduced. Equal parts of saliva and an aqueous solution of penicillin, 10,000 units per c.c., were mixed and allowed to stand for 15 minutes when 0.1 c.c. of the mixture was injected subcutaneously in the left inguinal region of pig No. 1. The same procedure was followed for pig No. 2 except that streptomycin solution, 100 mg. per c.c., was substituted for penicillin. Both animals reacted alike. Abscesses formed on the right side where untreated saliva was used. The sites of the saline and of the antibiotic-saliva mixture remained unchanged.

*Clinical Use*

Acute ulcerous Vincent's infection was selected for comparative observations. The lesions were confined to the gingivae. The patient rinsed the mouth with 5 ounces of tap water for one minute to remove debris. Cotton rolls and a saliva ejector isolated the maxillary and mandibular gingivae, and the tissues were dried with warm air. If the cotton rolls became satured with saliva, they were replaced. The medicament was applied with a glass syringe and a blunt curved gold-plated needle to all lesions, interdental spaces, and gingival sulci. The tissues were kept moist with the antibiotic for 15 minutes. While the quantity necessary for this process varies from 3 to 5 c.c. depending upon the size of the mouth, we used 5 c.c. in every case in order to standardize the procedure. The patient rinsed his mouth on completion of treatment. He received no instructions concerning smoking, diet, tooth brushing or home care.

Dark-field preparations were examined before treatment, at completion of treatment (15 minutes), 30 minutes, 60 minutes, and 24 hours. The average for 4 cases of penicillin and 4 cases of streptomycin therapy appear in Table I. Since it is impossible to count great numbers of microorganisms accurately or to measure the speed of motility, these observations are presented numerically. Countless numbers in all fields and normal motility are designated as 4. A few scattered organisms in an occasional field and spasmodic movements are recorded as 1. The intermediate values are 2 and 3. Naturally, there is considerable possibility of error in determining these intermediate values. Clinical observations were made of patients similarly treated as follows: With penicillin, 16 cases of Vincent's infection, 2 cases of pericoronitis; with streptomycin, 10 cases of Vincent's infection, 2 cases of pericoronitis.

All patients with Vincent's infection complained of pain, gingival tenderness, fetor oris, bleeding, salivation, and malaise. Interdental papillae were eroded with necrotic edges. The marginal gingiva was dark and edematous. Twenty-four hours after treatment, there was great amelioration in every instance. The tissues could be squeezed without acute discomfort, and solid food was chewed comfortably. Ulceration, fetor, and excess salivation had disap-

peared. The gingivae were lighter in color, although the margins and interdental papillae were red and edematous. Those patients treated with penicillin reported that relief from subjective symptoms appeared 4 to 12 hours after treatment. Streptomycin relieved the symptoms in 2 to 6 hours.

TABLE I

MOTILITY

| PENICILLIN | | | | STREPTOMYCIN | | | |
|---|---|---|---|---|---|---|---|
| TIME* | SP. | V | B | TIME* | SP. | V | B |
| 0 | 4 | 4 | 4 | 0 | 4 | 4 | 4 |
| 15 | 2-4 | 1 | 2 | 15 | 1 | 0 | 2 |
| 30 | 0-4 | 0 | 1 | 30 | 1 | 0 | 2 |
| 60 | 0-4 | 0 | 0 | 60 | 0 | 0 | 0 |
| 24 hrs. | 0-4 | 0 | 4 | 24 hrs. | 0 | 0 | 4 |

NUMBERS

| PENICILLIN | | | | | STREPTOMYCIN | | | | |
|---|---|---|---|---|---|---|---|---|---|
| TIME* | SP. | V | B | C | TIME* | SP. | V | B | C |
| 0 | 4 | 4 | 4 | 1 | 0 | 4 | 4 | 4 | 1 |
| 15 | 2 | 1 | 2 | 2 | 15 | 1 | 0 | 1 | 1 |
| 30 | 0-2 | 0 | 0 | 3 | 30 | 1 | 0 | 2 | 2 |
| 60 | 0-1 | 0 | 0 | 2 | 60 | 0 | 0 | 0 | 2 |
| 24 hrs. | 0-1 | 0 | 4 | 3 | 24 hrs. | 0-1 | 0 | 3 | 3 |

Sp. = spirochetes
V = vibrios
B = motile bacilli
C = cocci
*Time in minutes, except where indicated.

## DISCUSSION

Streptomycin acted more rapidly than penicillin. This is explained by the rapid penetration through surroundings which prevent penicillin from making prompt contact with the bacteria. Streptomycin is bactericidal during both the reproductive and resting stages of the bacterial cell,[6] while penicillin is bactericidal only during the reproductive phase.[7]

Penicillin can be introduced parenterally or by mouth as well as by topical applications. A limited number of uncomfortable oral reactions have followed all of these methods. The parenteral use of streptomycin may be accompanied by vertigo and stomatitis. No untoward effects have been reported from topical application. Neither antibiotic caused any changes in the teeth or in restorations.

## CONCLUSIONS

1. Both penicillin and streptomycin solutions in appropriate concentration are effective topically for treatment of the bacterial phases of acute Vincent's infection and pericoronitis.

2. Streptomycin is more rapid in action and can be stored for a longer time.

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# FURTHER STUDIES ON THE EFFECT OF HUMAN SALIVA ON THE CHOLERA VIBRIO IN VITRO

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## INTRODUCTION

IN THE recent cholera epidemic in Egypt, September through December, 1947, an apparent immunity existed whereby normal healthy individuals were comparatively free from the ravages of the infection although they were exposed to the same environmental conditions as those which were afflicted, the malnourished classes. It was these individual differences which led to the inquiry of the self-regulatory defense mechanism of the gastrointestinal tract, the first factor being saliva.

In previous experiments reported by the authors,[1] it was pointed out that human saliva from normal healthy individuals contained one or more agents which, under the conditions of the experiments, were capable of inhibiting the growth of, and lysing cholera vibrio in vitro. This activity could not be demonstrated to the same degree in the saliva from the cholera study group. The constancy of this action varied in the same individual at different times. Dissimilarities were observed between the antibacterial effectiveness against cholera vibrio and other pathogenic organisms. Kesteren, Bibby, and Berry[2] have previously shown evidence that there are several agents involved in the antibacterial effects of saliva.

Prior to further analysis of the relationship of saliva and its antibacterial properties, more detailed studies were carried on with the results reported here. To accomplish this objective, saliva samples from normal healthy males were subjected to various treatments and then tested against the cholera vibrio and other organisms. Continuous studies were made of its mode of action and attempts at concentration, purification, and biochemical analysis were undertaken. Finally, saliva samples from malnourished individuals of comparative age and sex were investigated in relation to the information gathered from the work on saliva from normal healthy subjects.

## MATERIALS

*Organisms.*—The cholera vibrio cultures used as the test organism were obtained by Dr. S. G. Rainsford* from stool specimens of an individual with acute symptoms of cholera infection. The strain was of the "Inaba" type and appeared to have the usual characteristics of the species.[3] The other organisms employed were *Bacterium paratyphosum A., Bact. paratyphosum B., Bact. paratyphosum C., Bact. coli communis; Shigella dysenteriae, Staphylococcus aureus,*

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*Streptococcus alpha,* and *Bacillus diphtheriae.* All were smooth and virulent strains. The bacteria were grown in pure cultures employing media suitable for the particular organism. The various organisms were seeded every twenty-four hours to eliminate the possible transition into mucoid variances. Test for the "salivary-effect" on the cholera vibrio were made on peptone agar and MacConkey's media.

*Saliva.*—The saliva used as test samples were collected by stimulative and nonstimulative methods from cholera patients, malnourished individuals, and normal healthy subjects, all of comparative age and sex.

## EXPERIMENTAL PROCEDURES AND RESULTS

Previous studies[1] have indicated that in order to determine more accurately the antibacterial nature and potency of human saliva on the test organism cholera vibrio in vitro by spectrophotometric methods, a more refined and controlled procedure would have to be developed. With this objective in mind the following procedure was evolved:

Saliva samples, 20 to 30 ml., were collected in sterile tubes and separately passed through an aluminum oxide chromatographic adsorption column. The column before use was activated by a weak acid or alkalid which necessitated restoring the filtrate to its original pH. This method of filtration removed bacteria, epithelial cells, and debris contained in saliva. As normal saliva is colorless, no chromatogram developed on the column. Thus to maintain the separation of the saliva accomplished by the aluminum oxide column, the filtrate was collected in 2 ml. amounts and tested for antibacterial activity by incubation with the test organism, cholera vibrio. After two hours, pour plates are made from each sample filtrate and read after twenty-four hours' incubation at 37° C. When the results were interpreted according to the antibacterial activity produced by each sample filtrate, this method of filtration proved to have the quality of separating whole saliva into three phases; complete inhibition, partial inhibition, and no inhibition.

In order to determine the potency of the active agents in saliva filtrates of normal healthy subjects and to obtain sufficient quantities of the antibiotic agents for bacteriological, chemical, and physical examinations it was necessary to collect the active portions in sufficient volumes. Samples of stimulated whole saliva from 20 normal healthy individuals were collected, pooled, and stored at 6° C. until sufficient quantities were obtained to make a volume of 5 liters. The pooled saliva was then passed through an activated aluminum oxide column which was prepared as follows: A hollow glass tube, three inches long, is fused to the bottom of a large test tube, 32 mm. × 200 mm. Glass wool is tightly packed to form a one-inch base and 100 Gm. of aluminum oxide is poured above the glass wool to form the body of the column (Fig. 1). The column is washed with 2 liters of distilled water and activated with 50 ml. of 0.01N HCl. After activation the column is again washed with 100 ml. of distilled water and placed in a drying oven, 140° F., for 12 hours. The column is washed, reactivated, and dried after each 400 ml. filtration. Vacuum applied at a negative pressure equal to 25 mm. of mercury accomplishes the filtration.

In order to facilitate the filtration of such a large volume as 5 liters, the filtrates were collected in 20 ml. amounts and tested for antibacterial activity against the test organism, cholera vibrio. Filtrates showing complete inhibition (henceforth referred to as "the active portions") were pooled and stored. The filtrate exhibiting partial or no inhibition were saved for refiltration (Table IA). After the 5 liters were filtered, the column was activated with

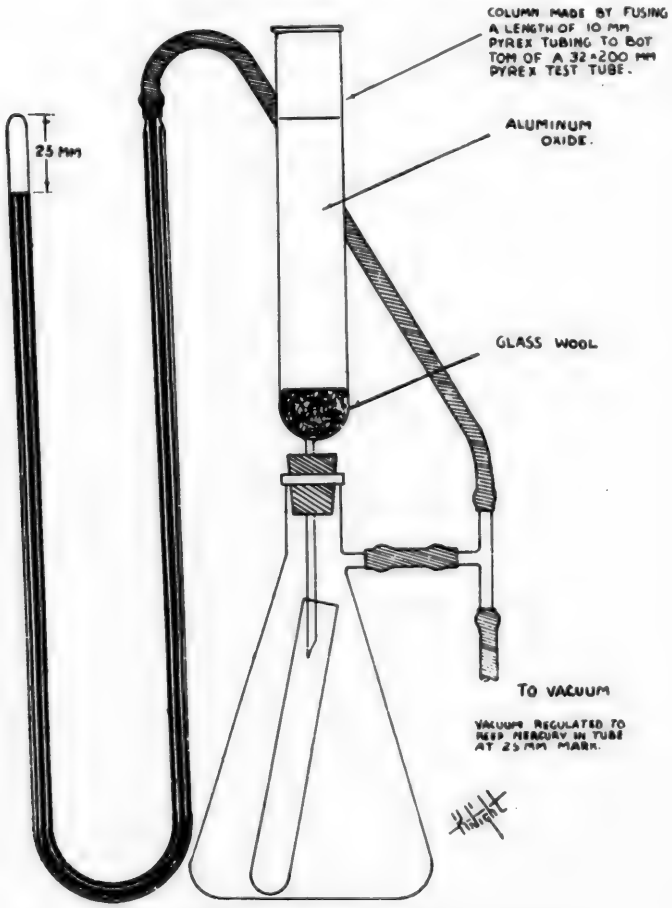
COLUMN MADE BY FUSING A LENGTH OF 10 MM PYREX TUBING TO BOTTOM OF A 32×200 MM PYREX TEST TUBE.
ALUMINUM OXIDE.
25 MM
GLASS WOOL
TO VACUUM
VACUUM REGULATED TO KEEP MERCURY IN TUBE AT 25 MM MARK.

Fig. 1.—Schematic drawing of adsorption column.

0.1N HCl and the filtrates showing partial or no inhibition were refiltered (Table IB). The filtered portions manifesting complete inhibition were pooled with the active filtrates obtained from the first filtration. The aluminum oxide column was then prepared for the filtration of the combined active portions by activating it with 1N HCl. The filtrates were collected in 5 ml. amounts and tested for inhibitory activity. After each 100 ml. volume, the column was washed, activated, and dried. As the tubes numbering 1 through 9 and 12 through 20

gave complete inhibition, the series of filtrates (1-9) were designated as the "first active principle," and the tubes numbering 12 through 20 the "second active principle." Tubes marked 10 and 11 consistently showed no inhibition (Table IC).

TABLE 1A

OCCURRENCE OF ACTIVE AND NONACTIVE PRINCIPLES IN SALIVA FROM NORMAL HEALTHY INDIVIDUALS AFTER FIRST FILTRATION THROUGH ALUMINUM OXIDE ADSORPTION COLUMN, ACTIVATED WITH 0.01N HCl

| PORTIONS FILTERED 400 ML. AMT. | | TUBE NUMBERS | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| No. | 1 | x | x | – | – | – | – | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | 2 | – | – | – | – | – | – | – | – | – | x | x | x | x | x | x | x | x | – | – | x |
| | 3 | x | – | – | – | – | – | – | x | x | x | x | x | x | – | – | – | – | – | – | – |
| | 4 | x | x | – | – | – | – | x | x | x | x | x | x | x | x | x | – | – | – | – | x |
| | 5 | x | x | – | – | – | x | x | x | x | x | x | x | x | – | – | – | – | – | – | x |
| | 6 | x | x | – | – | – | – | – | – | x | x | – | x | – | – | – | – | x | x | x | x |
| | 7 | – | – | – | – | – | – | – | x | x | x | x | – | – | – | – | – | x | x | x | x |
| | 8 | x | x | – | – | – | – | – | – | – | x | x | x | x | – | – | – | – | – | – | – |
| | 9 | x | – | – | – | – | – | – | x | x | x | x | x | x | – | – | – | – | – | – | x |
| | 10 | – | – | – | – | – | – | x | x | x | x | x | x | – | – | – | – | – | – | – | – |
| | 11 | x | x | x | – | – | – | – | – | – | x | x | x | x | x | x | – | – | – | – | x |
| | 12 | x | – | – | – | – | – | – | x | x | x | x | x | x | – | – | – | – | – | – | x |

X = Partial or no inhibition. — = Complete inhibition.

*Inhibitory and Lytic Activity.*—In order to determine the inhibitory and lytic action of the active principles, several procedures were employed. Inhibition was demonstrated by plating methods and the property to lyse by changes of turbidity in the bacterial suspension as recorded on a Beckman spectrophotometer. The tests were repeated a sufficient number of times to insure accuracy of the results. Samples from both the first and second active principles were tested against cholera vibrio and eight other bacteria.

TABLE 1B

OCCURRENCE OF ACTIVE AND NONACTIVE PRINCIPLES IN SALIVA FROM NORMAL HEALTHY INDIVIDUALS AFTER SECOND FILTRATION THROUGH ALUMINUM OXIDE ADSORPTION COLUMN, ACTIVATED WITH 0.1N HCl

| PORTIONS FILTERED 400 ML. AMT. | | TUBE NUMBERS | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| No. | 1 | x | x | – | – | – | x | x | x | x | x | x | x | x | x | x | x | – | – | – | x |
| | 2 | x | – | – | x | x | x | x | x | x | x | x | – | – | x | x | x | x | x | x | x |
| | 3 | x | x | x | x | x | – | – | – | – | – | x | x | x | x | x | x | x | x | x | x |
| | 4 | x | x | – | – | x | x | x | – | – | – | x | x | x | x | x | x | x | x | – | – |
| | 5 | x | x | – | – | x | x | x | x | x | x | x | x | x | x | x | x | – | – | – | – |
| | 6 | x | – | – | – | x | x | x | x | x | x | x | x | x | x | x | x | – | – | – | – |

X = Partial or no inhibition. — = Complete inhibition.

One milliliter of each active principle was mixed with 2 ml. of the bacterial suspension of the organism to be tested. The bacterial suspensions were grown on agar slants, washed off with 0.9 sterile saline, and diluted with 0.9 sterile saline to the optical density of 0.15 at a wave length of 500 millimicrons as read on a Coleman junior spectrophotometer. This optical density is comparable

TABLE IC

OCCURRENCE OF ACTIVE AND NONACTIVE PRINCIPLES IN SALIVA FROM NORMAL HEALTHY INDIVIDUALS AFTER THIRD FILTRATION THROUGH ALUMINUM OXIDE ADSORPTION COLUMN, ACTIVATED WITH 1N HCl

| PORTIONS FILTERED 100 ML. AMT. | | TUBE NUMBERS | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| No. | 1 | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – | – |
| | 2 | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – | – |
| | 3 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 4 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 5 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 6 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 7 | – | – | – | – | – | – | – | – | x | x | x | – | – | – | – | – | – | – | – | – |
| | 8 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 9 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 10 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 11 | – | – | – | – | – | – | – | – | – | x | x | x | – | – | – | – | – | – | – | – |
| | 12 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 13 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 14 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 15 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 16 | – | – | – | – | – | – | – | – | – | x | x | x | – | – | – | – | – | – | – | – |
| | 17 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 18 | – | – | – | – | – | – | – | – | x | x | x | – | – | – | – | – | – | – | – | – |
| | 19 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 20 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 21 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 22 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 23 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 24 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 25 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 26 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 27 | – | – | – | – | – | – | – | – | – | x | x | – | – | – | – | – | – | – | – | – |
| | 28 | – | – | – | – | – | – | – | – | – | x | x | x | – | – | – | – | – | – | – | – |

x = Partial or no inhibition. — = Complete inhibition.

to the McFarland barium sulfate standard[4] of 600 million bacteria. As a control, 1 ml. of 0.9 sterile saline was substituted for the first or second active sample.

Using a Beckman spectrophotometer, the test solutions were read at zero time and at thirty-minute intervals for five hours. A series of trials on measuring optical density at different wave lengths revealed that the optimum wave length was 350 millimicrons with a slit opening of 0.2 mm. This procedure thus measured the degree of lysis by the change in optical density (Fig. 2).

As a means of determining the inhibitory activity of the first and second active principles, pour plates were made at the end of the five-hour period with 0.1 ml. of the test sample using media suitable for the specific organism (Fig. 3).

Stimulated and Nonstimulated Saliva.—Nonstimulated whole saliva exhibited greater inhibitory power than stimulated whole saliva, although in contrast to this, no appreciable difference could be ascertained in their respective filtrates.

Heat.—Partial inhibition was obtained when 5 ml. of the first and second active principles were subjected to 75° C. for five minutes and then tested bacteriologically. After heating the samples for fifteen minutes at 100° C., the inhibitory power of both samples were destroyed.

Incubation.—Incubation at 37° C. for twenty-four hours had no apparent effect on the antibacterial activities of the test samples on cholera vibrio. Both active portions of saliva survived twelve days at room temperature without any appreciable change in potency.

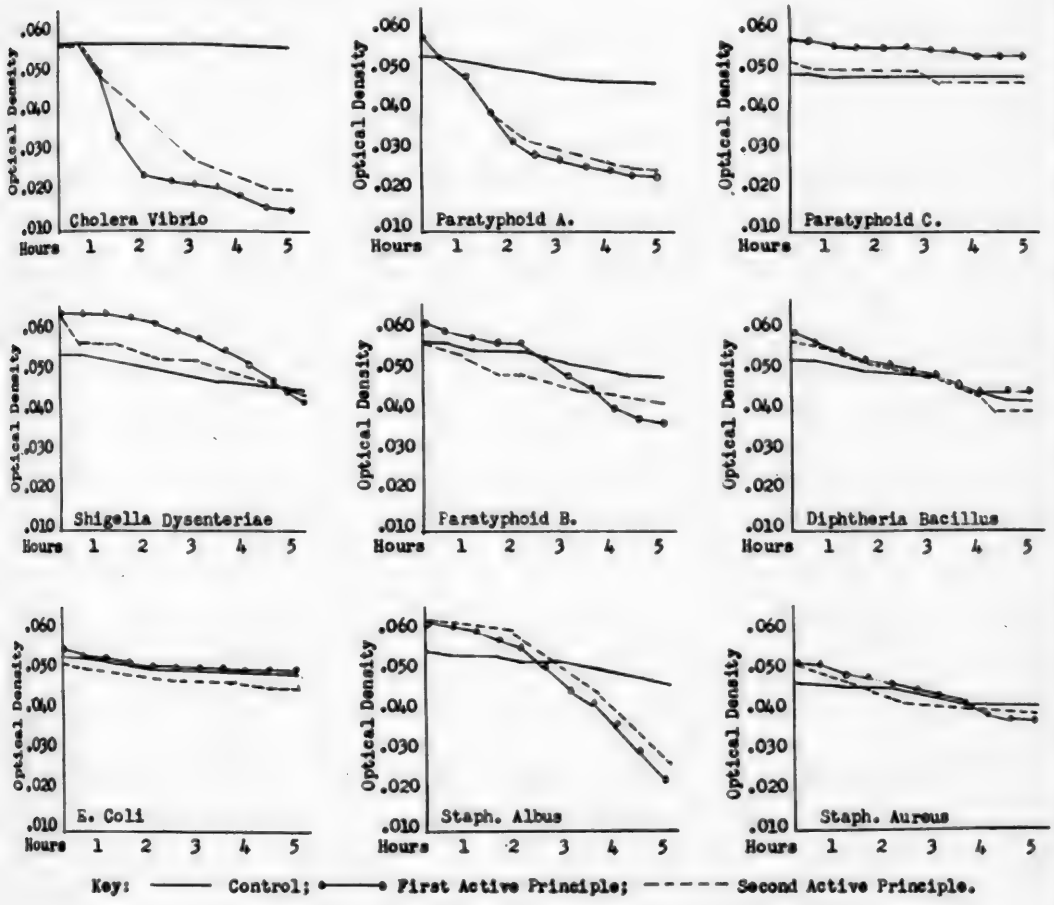


Fig. 2.—Comparison of the lytic power of saliva on various organisms.

Refrigeration.—When both samples of saliva were refrigerated at 6° C. for five months, or at 0° C. for twenty-four hours, neither samples gave any indication of a change in antibacterial properties.

Radiation.—Ultraviolet radiation of the two test filtrates at varying periods of time at a distance of sixteen inches did not reveal any antibiotic change during one to four hours. After four hours of exposure, there was a slight decrease in the lytic action of both samples, but no change in the bacteriostatic effect of either sample (Fig. 4).

Centrifugation.—Both active principles of saliva were centrifuged at varying periods of time and at various r.p.m's. There was no demonstrable changes in the samples' antibacterial actions.

Filtration.—When whole saliva was filtered through a Seitz K-5 clarifying pad, the filtrate exhibited bacteriostatic effect against the cholera vibrio. However, due to the presence of other bacteria of the oral flora which readily passed through the filter pad, there was no certainty that the bacteriostatic action was not influenced by bacterial antagonism. To eliminate this possibility a S-3 sterilizing pad was substituted for the K-5 clarifying pad. The sterile filtrate of whole saliva continued to show a bacteriostatic effect on cholera vibrio, although of a lower titer than that shown by the first or second active principles obtained by filtering through the aluminum oxide column.

Effect of pH.—To determine the influence of changes in pH, two portions, 25 ml. each, of whole saliva from a normal healthy individual were filtered through separate aluminum oxide columns. One column was activated by 1N HCl and the other by 1N NaOH. Neither column was washed after activation. The filtrates were collected in 5 ml. amounts and the pH checked on a Beckman pH meter. As each sample varied in pH, the latter samples approaching the pH of normal saliva, they were tested for their bacteriostatic effect by the aforementioned culture-plate method using cholera vibrio as the test organism. It was found that an acidity of pH 3.1 and an alkalinity of pH 11.3 did not alter the inhibitory effect of the two active principles of saliva, for in the absence of the active principles cholera vibrio was not inhibited.

TABLE II

PRECIPITATION RESULTS OF WHOLE AND FILTERED SALIVA WITH VARIOUS CHEMICAL REAGENTS

| CHEMICALS USED | FIRST PRINCIPLE | SECOND PRINCIPLE | WHOLE SALIVA |
|---|---|---|---|
| Hydrochloric acid | Neg. | Neg. | Pos. |
| Acetone | Neg. | Neg. | Pos. |
| Nitric acid | Neg. | Neg. | Pos. |
| Sodium hydroxide | Neg. | Neg. | Pos. |
| Alcohol, 95 per cent | Neg. | Neg. | Pos. |
| Chloroform | Neg. | Neg. | Pos. |
| Ether | Neg. | Neg. | Pos. |
| Trichloracetic acid | Neg. | Neg. | Pos. |
| Sulphuric acid | Neg. | Neg. | Pos. |
| Sodium tungstate | Neg. | Neg. | Pos. |
| Phosphoric acid | Neg. | Neg. | Pos. |

Precipitation.—Tests were conducted with various chemical agents to determine the properties of whole saliva and those of the first and second active principles. It proved to be impossible to precipitate the first or second active principles with the agents used; on the other hand, whole saliva was precipitated by all the reagents (Table II).

Adsorption.—When the two active principles were tested for adsorptive properties on activated charcoal, kaolin, and aluminum oxide, the results revealed that only charcoal adsorbed both active principles. The kaolin and aluminum oxide exhibited no property of adsorption.

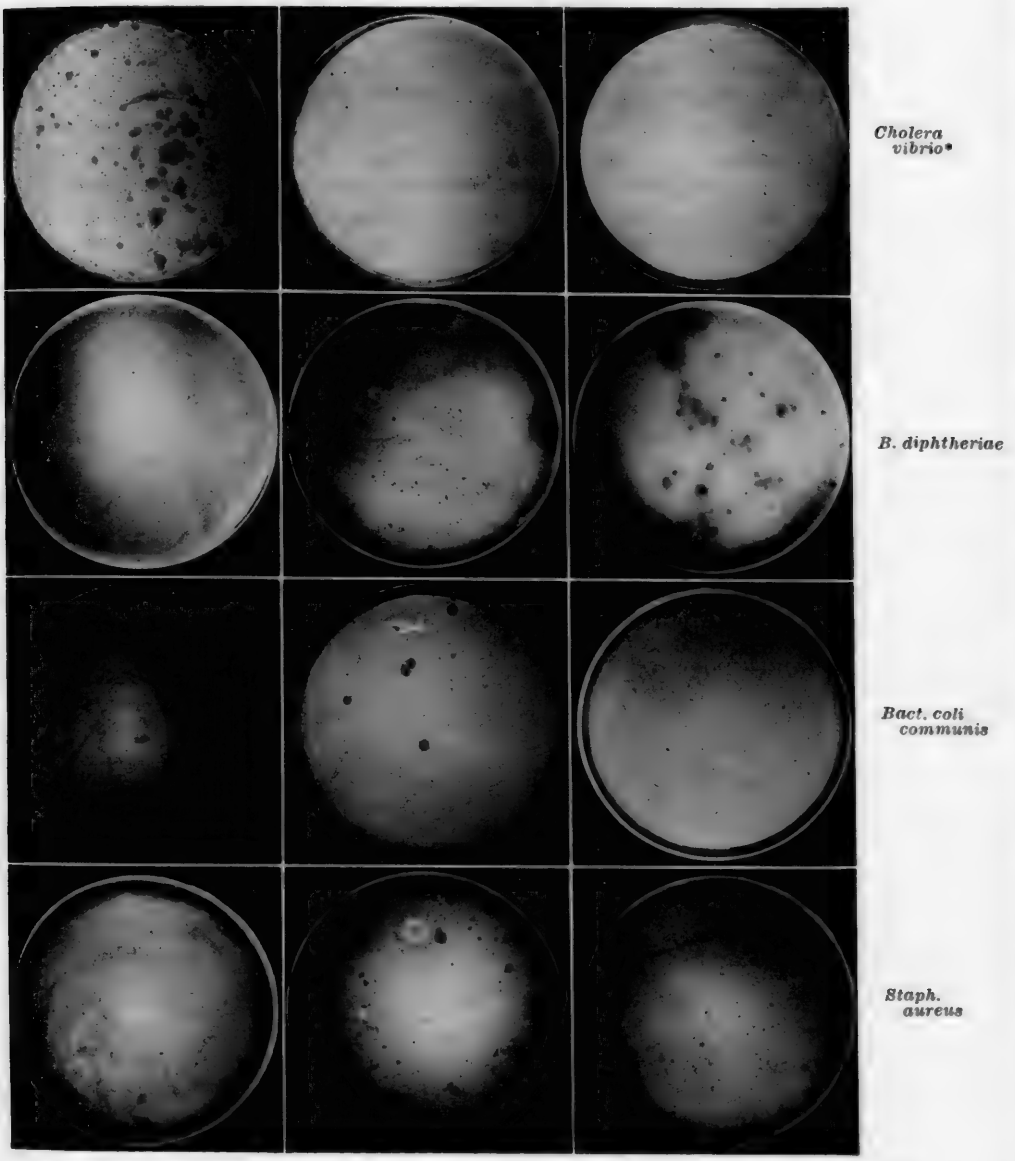


Fig. 3.—Inhibitory activity of the first and second active principles of saliva on various organisms.

*Organisms also exhibiting complete inhibition were *Bact. paratyphosum A, B, C,* and *Shigella dysenteriae.* Note specificity of the first active principle on *Bact. coli communis.*

Test for Protein.—As no precipitate was formed by the agents commonly used to precipitate protein, the biuret test[5] for proteins was accomplished. Similarly, both active principles of saliva gave negative results.

Test for Amylase.—The Fabicus-Moller[4] qualitative and the Somolgi[4] quantitative tests for amylase were done on both active principles with negative results. Whole saliva was positive for amylase.

Test for Carbohydrase.—Since the inhibitory action could not be attributed to a demonstrable protein, it was further investigated by determining if a carbohydrase was present. A series of experiments were done whereby twelve different sugars were inoculated with inoculum from both active principles of saliva. After ten days' incubation neither gas or acid were produced.

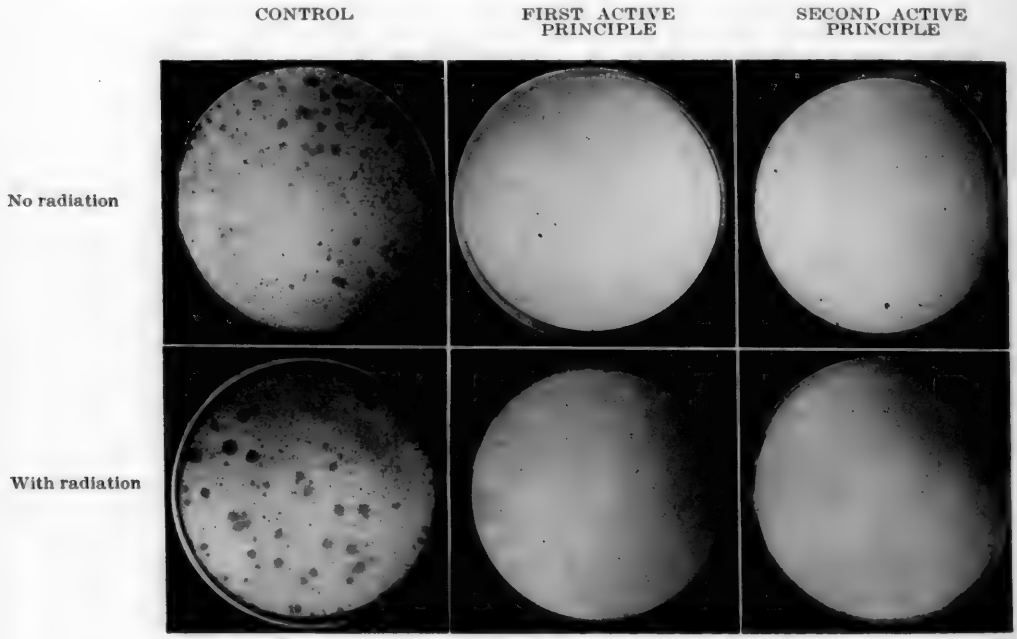


Fig. 4.—Effects of five hours' ultraviolet exposure of the first and second active principles of normal saliva on cholera vibrio. Note there has been complete inhibition of growth.

Test for Urea.—Karr's[4] method for the determination of urea gave negative results in the samples of saliva filtrates, but gave positive indication that it was present in the whole saliva samples.

Test for Cholesterol.—The presence of cholesterol could not be demonstrated in either of the active principles. Whole saliva gave a normal positive reaction.

Test for Lipase.—As no evidence could be shown with the procedures used that the inhibitory agent or agents were due to a protease or a carbohydrase, the series of experiments were continued to determine the presence of lipase. One milliliter of each active principle of saliva was added to a 5 per cent suspension

of R. B.C's. in physiological salt solution. After one hour at 75° F., both showed complete hemolysis of the R.B.C's indicating that a lipase may have been present. Subsequent tests prove this to be true. A qualitative test was carried out by incubating 1 ml. of each active principle with an emulsion of olive oil in water containing gum acacia. The fatty acid liberated was titrated with 0.05N NaOH employing phenolphthalein as a color indicator. The first active principle gave an average titration value of 0.25 ml. The second active principle gave an average of 0.42 ml. When the samples of saliva containing the active principle were tested quantitatively for lipid phosphorous (lecithin)[5] the active principles contained an average of 2.6 and 2.9 mg./100 ml. of filtrate respectively.

It has been previously seen[1] that vitro experiments on the cholera vibrio revealed a marked discrepancy in the antibacterial effect of saliva from normal healthy individuals to that which was obtained from patients suffering from cholera. It was also observed that in epidemic-type cholera, the persons with symptoms of the disease were generally restricted to those in a malnourished condition. Since a lipase was the only agent demonstrable by the aforementioned procedures and the important role lipases play physiologically,[8] it was of interest to determine the relationship if any, of this lipase in saliva and other body fluids of malnourished subjects to that of normal healthy controls.

Stimulated saliva from 10 malnourished individuals were collected in 25 ml. samples and filtered through an aluminum oxide column. Saliva from 10 normal healthy individuals collected during the same period were used as controls.

TABLE III

COMPARISON OF PHOSPHOLIPID CONTENT IN SALIVA AND PLASMA OF HEALTHY AND MALNOURISHED TEST GROUPS

| DIFFERENT SALIVAS | PLASMA HEALTHY CONTROL IN MG./100 ML. | PLASMA MALNOURISHED IN MG./100 ML. | SALIVA FILTRATE HEALTHY CONTROL IN MG./100 ML. | SALIVA FILTRATE MALNOURISHED IN MG./100 ML. |
|---|---|---|---|---|
| A | 7.5 | 5.8 | 2.0 | 0.7 |
| B | 7.0 | 3.9 | 1.7 | Neg. |
| C | 6.4 | 5.0 | 1.2 | 0.6 |
| D | 6.5 | 4.8 | 1.4 | 0.5 |
| E | 8.0 | 4.5 | 2.1 | 0.4 |
| F | 7.8 | 4.7 | 2.0 | 0.5 |
| G | 7.2 | 4.2 | 1.8 | 0.3 |
| H | 7.4 | 4.9 | 1.8 | 0.6 |
| I | 6.9 | 5.3 | 1.6 | 0.7 |
| J | 7.5 | 4.4 | 1.9 | 0.5 |
| Tests average | 7.22 | 4.75 | 1.75 | 0.48 |

The saliva filtrates were collected in one tube and not concentrated into active and nonactive portions. Ten milliliters of oxalated blood were collected from each group at the same time as the saliva samples. The saliva filtrates and the plasma were tested for their lipid phosphorus content. In no instance was the lipid phosphorus content of saliva and plasma from the malnourished group as high as that found in the control group (Table III). Saliva samples from the malnourished group was then studied in relation to their lytic power and inhibition of the cholera vibrio. The saliva from the normal healthy group served as controls. The spectrophotometric procedure previously outlined was

used to determine the degree of lysis. The saliva filtrates from the malnourished group showed little or no lysis in contrast to the filtrates of the control group which demonstrated considerable lysis (Fig. 5). In addition, the filtrates from the malnourished group exhibited no bacteriostatic action on the test organism (Fig. 6).

## DISCUSSION

The experiments described in the present paper substantiate and augment preliminary studies, cited in the Introduction, which described an agent, or agents, in saliva from normal healthy individuals which were capable of lysing and inhibiting the growth of cholera vibrio in vitro. The findings also confirmed the ineffectiveness of saliva from cholera infected persons. The results of this study, in addition, tend to indicate that differences in potency do exist in saliva from the healthy and malnourished individuals.

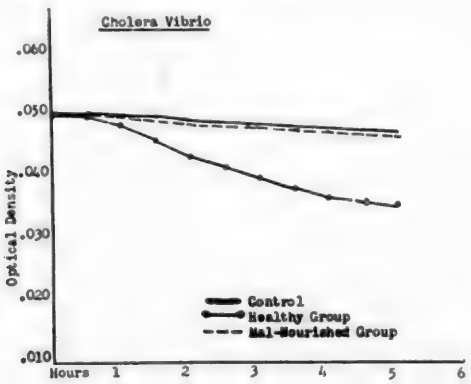


Fig. 5.—Average lytic power of salvia filtrates from healthy and malnourished test groups.

In discussing cholera as seen in Egypt during the past epidemic, consideration of the relation of undernourished individuals and their susceptibility to contract cholera revealed a close and striking parallel. The "Inaba" type was the predominate strain isolated, and the active principles of saliva from the healthy group were effective in lysing and in inhibiting it. Tests on the "Rangoon" strain indicated that the active principles were equally as effective. Unfortunately, the "Ogawa" strain was not available for investigation.

Several considerations entered into the development of the technic for obtaining saliva filtrates as described in these studies. Adsorption at a suitable pH with aluminum oxide offers certain advantages over other filtration methods generally employed such as the Seitz or Berkfield. The adsorption method consists of sucking whole saliva through a column of some suitable adsorbent. Depending upon the differences in the tendency to become adsorbed, influenced by the pH of the column, individual constituents of saliva are adsorbed. This method eliminates the discrepancies encountered from the various components

of whole saliva. The collection of the saliva filtrates in 2 ml. or in smaller amounts, permits one to concentrate and separate the active and nonactive portions.

One of the major weaknesses in the work on saliva is in the methods used to determine bacteriolytic and bacteriostatic activity. Visual clearing of opaque solutions of bacterial suspensions,[7] or measurement of the transparent zones of inhibition around saliva wells in a lightly inoculated pour plate,[6] do not permit accurate differentiation between lytic or inhibitory activity. On the other hand, using the spectrophotometric procedure in conjunction with a modified plating technic, lysis and inhibition can be differentiated. The lytic action can be recorded accurately and followed continuously by changes in optical density of the test suspension. It has been clearly shown (Fig. 3), that inhibition and lysis do not always take place synonymously, for the action may be either bacteriolytic, bacteriostatic, or a combination of both. The spectrophotometric technic for determination of bacterial lysis has proven to be accurate, rapid, and sensitive to minute change in optical density not apparent to the naked eye. The choice of the spectrophotometer was the Beckman quartz type, as a less sensitive instrument measures only the gross changes in optical density.

The adsorption method yielded two active principles. The outstanding difference between these filtrates was evidenced by their antibacterial specificity when tested against similar organisms (Fig. 2).

The saliva filtrates failed to maintain their maximum potency over a long period unless kept in a sterile condition and at a low temperature. When stored at 6° C., and in a sterile condition, they retained their potency almost indefinitely. After 75° C., the rate of lysis decreased proportionally to the rise in temperature. The activity was destroyed at 100° C.

Contrary to whole saliva, the lytic action of the active principles were not increased by 700 r.p.m. centrifugation. In contrast to the antibacterial properties of whole saliva which are destroyed at a r.p.m. above 1700 for two hours, the active principles of saliva filtrates were not affected. This latter property may possibly be due to the absence of any appreciable amount of protein (mucin).

We are at a loss to explain the stability of the active principles to changes in pH varying from 3.1 to 11.3. One would have anticipated the activity to be in a more limited range, that is, approximately that of normal saliva.

When considering the results of the attempts to form precipitates from whole saliva and saliva filtrates, the inability of saliva filtrates to yield a precipitate has been attributed to the removal of all the mucin by the aluminum oxide column (Table II).

The observed increase in bacteriostatic action of the saliva filtrates over that of whole saliva were apparently due to the elimination of the nonactive portions as separated by the aluminum oxide column. Thus there is a greater concentration of the active principles by volume to that of whole saliva.

SALIVA FILTRATES

CONTROL

Normal healthy group

Malnourished group

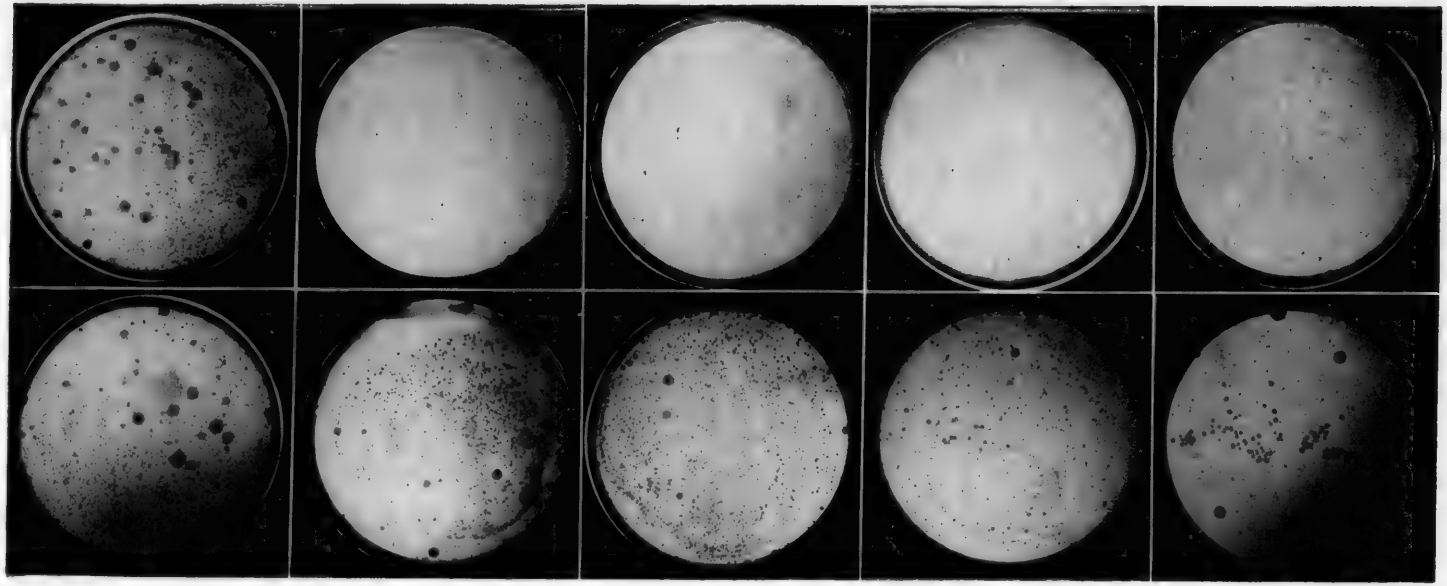

Fig. 6.—Representative pour plates showing the effects of saliva filtrates from healthy and malnourished individuals on cholera vibrio. Note slight or no bacteriostatic action of the malnourished group.

Obviously, further work is needed on the biochemical aspects of the active principles before their exact nature can be determined. The procedures accomplished by the authors were, at best, a feeble attempt. However, the results did reveal that phospholipids were present in larger concentration in saliva filtrates from the normal healthy group. This difference in saliva phospholipid may be an indication of the state of body nutrition. On the other hand, though no evidence has been shown, the active principles of saliva may be activated by an optimum concentration of phospholipid.

In man, we find cholera is contracted by ingestion of cholera organisms with water, food, or any contaminated material. The disease is essentially an intestinal one. It is believed possible that the bacteria, if in small numbers, may be checked by normal healthy saliva[1] and then by the gastric secretions. Thus the first line of defense is human saliva. Bacteria unharmed by saliva or gastric secretions pass into the intestines where in the absence of antibacterial activity of intestinal secretions they proliferate rapidly, producing the symptoms of acute cholera infection. It is assumed, although more investigations are necessary for adequate proof, that the intestinal secretions of normal healthy individuals have sufficient antibacterial properties which prevent this abundant proliferation of the cholera vibrio. The irritation and sloughing of the intestinal mucosa is believed to be the result of the toxin produced by the invading organism. Whether the host will overcome the infection, become a carrier, develop mild symptoms, or manifest acute symptoms depends upon the number and virulence of vibrio and the bacteriostatic effectiveness of the intestinal secretions.

Although the antibacterial effect of saliva has been described by other investigators[7] as being less effective than most other body secretions, it might well serve as an indicator of the antibacterial threshold of the other body secretions. Thus a high salivary titer would indicate a correspondingly high antibacterial titer in intestinal secretions. The low salivary titer found in the malnourished and cholera-infected group may, in part, explain why epidemic cholera is limited to the malnourished class of people. Provided that this premise is true, then social and economic difficulties more than medical must be overcome before cholera can be eradicated.

A valid criticism of this paper is that in vitro findings do not necessarily follow the in vivo effect. However, the effects demonstrated in vitro cannot be ignored until they have been proven not to operate in human secretions in the body.

## SUMMARY

Under the conditions of the experiments, saliva from normal healthy individuals was found to have two antibacterial agents. These agents varied in their antibacterial specificity and exhibited both bacteriolytic and bacteriostatic properties against the cholera vibrio and several other organisms which were tested. The agents appeared to be components of salivary secretions and to be enzymic in nature. The results indicated that there was more constancy in the antibacterial action of saliva filtrates than that of whole saliva. Saliva filtrates

from malnourished individuals and cholera patients revealed little or no antibacterial effect. This seems to indicate that the antibacterial properties of body secretions may play a dominant role in natural immunity to cholera infection.

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# INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

## Scientific Proceedings of the Philadelphia Section

### Temple University School of Dentistry, Nov. 21, 1949*

S. Leonard Rosenthal, *Editor, Philadelphia Section*

1. Changes Produced by X-rays in Metastatic Carcinoma of the Neck. *J. A. Donnelly, Temple University, Philadelphia, Pa.* Cervical lymph nodes involved by metastasis from oral lesions were treated by roentgen ray using high dosage over a small area. Removal and section of such nodes demonstrated partial or complete keratinization of the invading cells in the majority of the nodes examined.

2. Ammoniated Dentifrices—A Preliminary Bacteriologic Study. *H. M. Cobe, Temple University, Philadelphia, Pa.* High urea, low urea, quaternary compound, and cosmetic pastes and powders were studied in vitro and in vivo. Three hundred children, 10 to 14 years old, were placed under observation for 18 months. Groups were given pastes and powders containing: (1) high percentage urea, (2) low percentage urea, (3) a quaternary ammonium compound, and (4) no ammonium compound. *Lactobacillus acidophilus* counts revealed an average decrease of 46 per cent in the first 3 groups and an increase in Group 4. The greatest decrease followed the use of quaternary ammonium compound; the next greatest was the high urea dentifrice group. Powders were 8 per cent better than pastes.

3. The Dental Aptitude Testing Program. *R. Baralt, Temple University, Philadelphia, Pa.* The performance of students in course seems comparable to their score in the aptitude tests. The closest correlation was that of the carving test and the technic courses in operative dentistry. One hundred seven third year students repeated the carving test that they had taken on entrance, and the two tests were compared. One hundred students had a difference of 4 or less between their two scores. Of the entire group, 49 obtained a slightly higher score in the second test; 46 had a slightly lower score; the remainder received the same score. The carving test appears to be a test of innate dexterity rather than acquired skill.

Received for publication, November 28, 1949.
*Ernest Ritsert, presiding.

# INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

## Scientific Proceedings of the Pacific Northwest Section

University of Oregon Dental School, December 4, 1949

B. O. A. Thomas, *Editor, Pacific Northwest Section*

1. Transplantation and reimplantation of teeth. *Horace M. Miller, D.M.D., University of Oregon Dental School, Portland, Ore.* The history of transplantation and reimplantation of teeth was traced from 1590 to the present. Case histories of transplantation and reimplantation with roentgenograms taken before, immediately after, and after a series of years were presented. The study is a progressive experiment which is expected to cover three or four more years. Some cases presented were of over twenty-seven years' standing.

2. Registration of vertical intermaxillary force by the Brinell hardness method. *Claude Baker, D.D.S., University of Washington, School of Dentistry, Seattle, Wash.* Some of the instruments used at the present time for registering vertical intermaxillary forces require excessive opening of the jaws, thus distorting the intermaxillary relationship. A simple instrument was described which uses a 2 mm. steel ball for indenting a thin rolled sheet of aluminum of constant Brinell hardness.

3. Changes in width dimension of certain teeth and facial points during human growth. *Guy A. Woods, Jr., D.D.S., M.S., University of Oregon Dental School, Portland, Ore.* A serial study was made of 26 individuals between the ages of 3 and 15. The method used was oriented frontal and lateral cephalometric films which were corrected by the use of the cephalometric compensator developed by Drs. Wylie and Elsasser. The measurements taken were bizygometic; bigonial; intercanine, upper and lower; inter first molar, upper and lower.

4. Failure of the ammonium ion to inhibit growth of lactobacilli. *H. C. Douglas, Ph.D., W. F. Kirchheimer, M.D., Ph.D., R. H. Layton, B.S., University of Washington, Department of Microbiology, Seattle, Wash.* Definite laboratory evidence as presented to prove that several strains of lactobacilli are neither destroyed nor greatly inhibited in their growth by the addition of high concentrations of ammonium ion to the culture media. This is in direct contrast to the results reported by Kesel and associates at the University of Illinois. This important subject should be investigated further from both the laboratory and clinical aspects.

5. Comparative photography in dental science. *Jack De Ment and Russell Culbertson, University of Oregon Dental School, Portland, Ore.* By use of simple changes in (a) film, (b) light source, and (c) filters, the inherent values of the camera can be extended at least twofold. Comparative photography in dental science involves, for example, supplementing the visible light photograph with (1) infrared, (2) ultraviolet, and (3) fluorescence photographs. Structures which are not visible by white light are often very evident in one or more of the supplemental photographs. Photography in pure (invisible) ultraviolet light reveals surface structure in exceptional detail. Wider use and appreciation of these methods is advocated in dental research and education.